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Synthesis and Kinetic Evaluation of Substrate-based Phospholipid Analogues and Studies Towards the Synthesis of 5-Hydroxyaloin A

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Synthesis and Kinetic Evaluation of Substrate-based Phospholipid Analogues and Studies Towards the Synthesis of 5-Hydroxyaloin A

by

Hui Li, B.S.; M.S.

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Dedication

To my mom, for everything she did.

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Synthesis and Kinetic Evaluation of Substrate-based Phospholipid Analogues and Studies Towards the Synthesis of 5-Hydroxyaloin A

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Supervisor: Stephen F. Martin

In the studies to establish structure-reactivity relationships in the hydrolytic reaction of phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidyl L-serine (PS) catalyzed by the phospholipase C from *Bacillus cereus* (PC-PLC$_{Bc}$), a number of water-soluble, non-hydrolyzable substrate based inhibitors of PLC$_{Bc}$ were prepared. These include ω-hydroxy phosphorodithioates and phosphonates of choline, ethanolamine and L-serine. Kinetic assays reveal that all the ω-hydroxy phosphorodithioates are good inhibitors with good aqueous solubilities. However, ethanolamine and L-serine derivatives of phosphonates fail to inhibit PLC$_{Bc}$ at their maximum solubilities. The three-dimensional structures of phosphonate-PS with E4G, E4Q and wild type PC-PLC$_{Bc}$ revealed that these mutants bind the PS analogue in a very different manner than wild-type does a PC analogue. The structural difference shed new views on our understanding of the mechanistic and kinetic aspects of PLC$_{Bc}$ catalyzed hydrolytic reactions.

In the application of our glycosyl furan/benzyne cycloaddition methodology towards natural product synthesis, a two-stage benzyne/furan cycloaddition strategy was used to assemble the anthrone core of Group I *C*-aryl glycoside 5-hydroxyaloin A. Proof of concept was established in the generation of benzyne from a chloronaphthol precursor **4.142** and subsequent cycloaddition with furan afford the cycloadduct **4.143**. However, the cycloadducts of **4.142** and alkoxy/silyloxy furans were unstable, and attempts to convert them into 5-hydroxyaloin A were unsuccessful. During the course of the investigation, cycloadditions using glycal-substituted furans were investigated and a one-step novel approach to the *C*-aryl glycal was established starting from 2-deoxy sugar lactone.

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Chapter 1: Phospholipase C and Phospholipid Analogues

The primary goal of this project is to establish structure-reactivity relationships in the hydrolytic reaction of phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidyl L-serine (PS) catalyzed by the phospholipase C from *B. cereus* (PLC_{Bc}). This chapter serves as an introduction to PLC_{Bc} and phospholipid synthesis, starting with the biology of phospholipids and phospholipases in general, followed by a description of the structural and mechanistic aspects of PLC_{Bc}. This chapter concludes with the chemical synthesis of phospholipids and their analogs since substrate mimics are important tools that were used in our group to elucidate the basis of the kinetic and thermodynamic differences.

1.1 PHOSPHOLIPIDS AND SIGNAL TRANSDUCTION

Biological membranes are lipid bilayers that form the barrier between the cell interior and its external milieu. They are also involved in cell compartmentalization to delimit cells and to divide the cytoplasm into its organelles and compartments. Mammalian cells contain a great variety of complex lipids. Phosphatidylcholine (PC), phosphatidylethanolamine (PE) and sphingomyelin are quantitatively the most abundant lipids in biological membranes (Figure 1.1). For decades, it has been thought these phospholipids serve only structural roles responsible for maintaining the integrity of cellular membrane. The discovery that the breakdown products of cellular phospholipids actively participate in biological processes and as such can interfere with cell physiology and pathology in the 80's opened a new field of research.

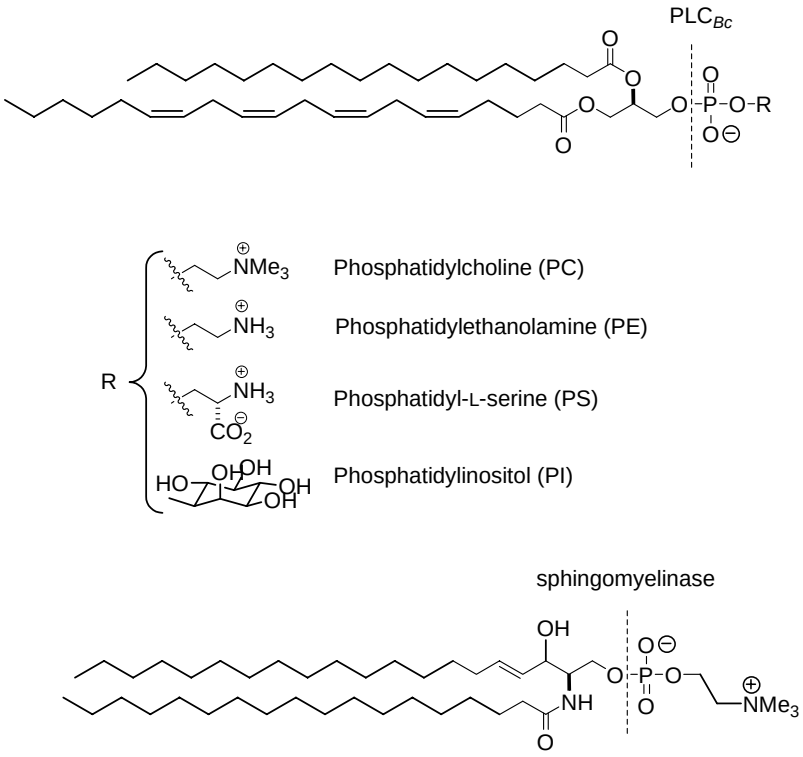


Figure 1.1: Common membrane phospholipids

Since then, the role of the membrane integral PLC class of enzymes in the cell signal transduction pathway has been studied extensively. In particular, extensive efforts have been devoted to the inositol specific phospholipase C (PI-PLC) because of its key role in initialing cellular response.[1-3] Activation of a hormone-sensitive PI-PLC at the plasma membrane results in rapid catabolism of the polyphosphoinositides to form the two secondary messengers inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). The initially formed water-soluble inositol 1,4,5-trisphosphate (IP_3) acts upon Ca^{2+} storage, resulting in a transient intracellular calcium concentration spike. However, IP_3 is rapidly metabolized by the action of a specific phosphatase *in vivo* through the removal of 5-phosphate, and the Ca^{2+} concentration in most tissue cells therefore returns rapidly to basal or even below basal levels.[4, 5] The other hydrolytic product, diacylglycerol (DAG)

remains in the plasma membrane and activates protein kinase C (PKC) (Figure 1.2). DAG dramatically increases the affinity of protein kinase C for Ca^{2+} and thereby renders it active in the presence of micromolar concentration of Ca^{2+}.[6, 7]

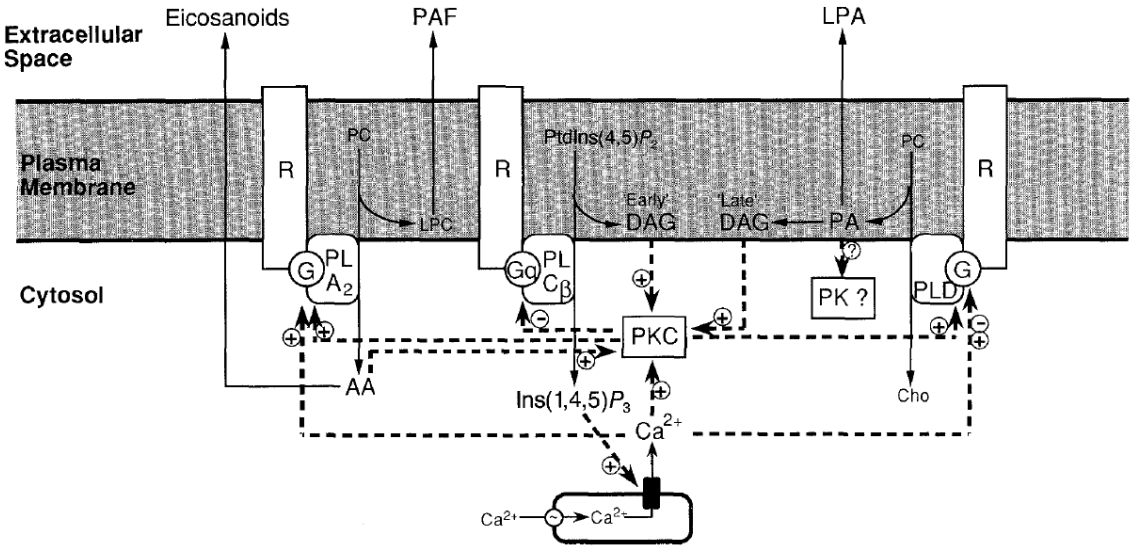


Figure 1.2: A schematic and simplified representation of receptor-mediated activation of multiple phospholipases in signal transduction. Note that all of the SAPs depicted are not necessarily occur in one particular cell. only G protein-mediated receptor-phospholipase coupling is depicted. Abbreviations: DAG, diacylglycerol; Cho, choline; Ins, D-myo-inositol; LPA, lysophosphatidic acid; LPC, lysophosphatidylcholine; PA, phosphatidic acid; PAF: platelet activating factor; PKC, protein kinase C; PIP2, phosphatidylinositol-4,5-diphosphate.[8]

Isotope labeling studies indicate that DAG is metabolized very rapidly by a group of enzymes both when it is produced endogenously and when it is added exogenously. It is either converted back to inositol phospholipids by way of phosphatidic acid (PI turnover) or to its further degradation product, *cis* unsaturated fatty acids, such as arachidonic acids. Consequently, the burst of membrane DAG concentration and cytosolic calcium concentration due to hydrolysis of phosphoinositides is rather short lived.

Recent experiments have established that sustained PKC activation is essential for long-term cellular response such as gene expression, cell proliferation and differentiation. Asaoka and Aihara demonstrated that a single dose of a membrane-permeable diacylglycerol (DAG), 1,2-dioctanoylglycerol (1,2-DiC8), was insufficient to allow HL-60 cells to differentiate to macrophages due to its rapid metabolism. However, either a single dose of the slowly-metabolized phorbol 12-myristate 13-acetate (PMA)[9] or an increase in the duration of exposure of HL-60 cells to 1,2-DiC8 by multiple additions greatly enhanced their differentiation to macrophages.[10] Similar phenomena were also observed in the activation of resting T lymphocytes.[11]

The sustained activation of PKC for a certain period of time is required for long-term cellular responses; however, the biochemical mechanism prolonging this enzyme activation under physiological conditions are not fully known. The formation of DAG by the mechanism of inositol phospholipid hydrolysis is transient and appears to be insufficient. The sustained elevation of cellular diacylglycerol concentration required for continuing activation of PKC accompanying with basal Ca^{2+} concentration in the relative later phase in cell response must therefore come from other hydrolytic pathways. Analysis of fatty acid compositions in cell membranes has indicated that the second phase of DAG formation probably results from hydrolysis of the more abundant phosphatidylcholine. Phosphatidylethanolamine appears to be a minor source of the diacylglycerol.

1.2 PHOSPHATIDYL CHOLINE-PREFERRING PHOSPHOLIPASE C FROM *BACILLUS CEREUS*

1.2.1 Overview of Phospholipases

Signal-activated enzymatic hydrolysis of phosphatidylcholine involves three distinct enzymes: phospholipase A_2 (PLA_2), phospholipase C (PLC), and phospholipase D (PLD). These phospholipases are categorized by the hydrolytic site (Figure 1.3)

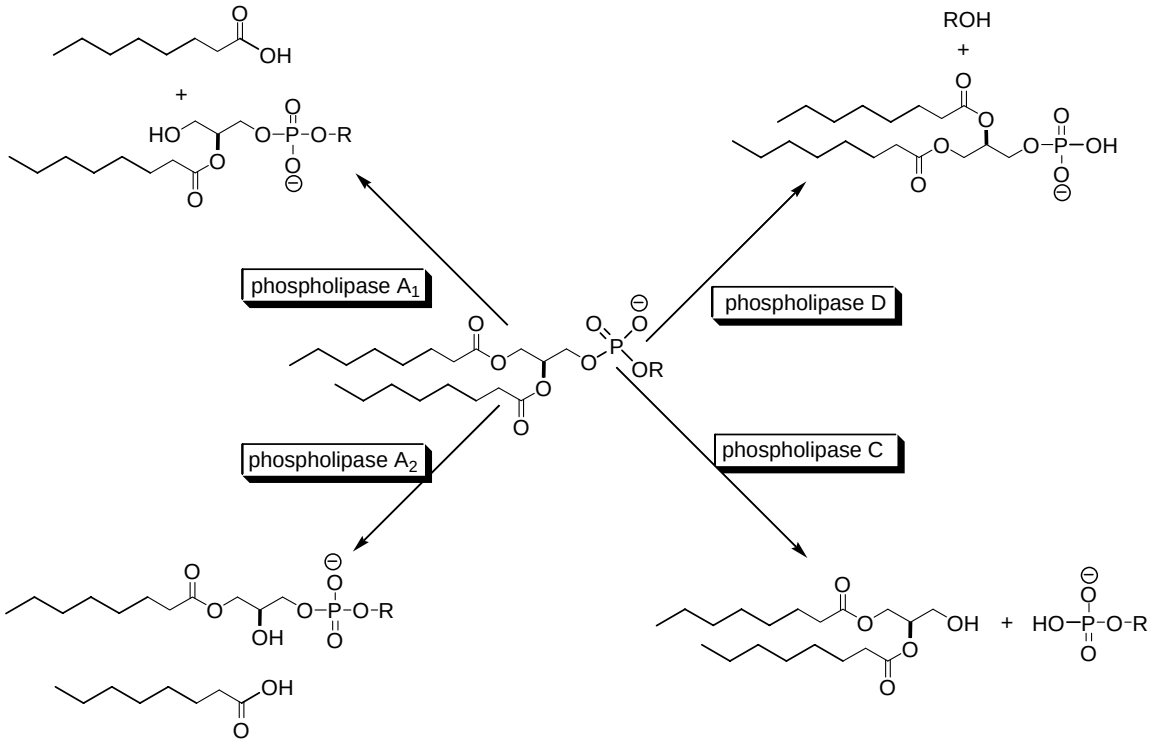


Figure 1.3: Phospholipase and their corresponding hydrolytic sites

Phospholipase A_2 catalyzes the release of fatty acid from the *sn*-2 position of phospholipids with concurrent formation of a lysophospholipid. The hydrolyzed product, *cis* unsaturated fatty acids including oleic, linoleic, linolenic, arachidonic acids activate PKC to various degrees. The PLA_2s that hydrolyze PC and PE occurs in both extracellular and intracellular forms. The former are found in secretory granules, snake

and bee venoms and pancreatic secretion. They are of low molecular weight and are believed not to be involved in cell signal transduction. The latter are found in plasma and intracellular membranes and also cytosol. Numerous extracellular phospholipase A_2 isoenzymes and their mutants from various sources, such as snake venom,[12-16] mammalian[17-19] and human[20-22] have been purified and crystal structures in native form and complexed with inhibitors have been obtained. Cytosolic PLA_2s from several sources have also been purified[23-26] and cloned,[27-31] and structurally established.[32, 33]

Phosphatidylcholine-specific phospholipase D (PLD) catalyzes the generation of choline and a second messenger phosphatidic acid, which acts directly as a signaling molecule.[34, 35] But the major metabolic fate of phosphatidic acid is phosphatase-mediated dephosphorylation to form diacylglycerol,[36] which represents an alternative pathway for DAG formation. PLD activity was first identified in plants, and it was the first PC-hydrolysing enzyme purified and successfully cloned.[37] The cloning of the first plant PLD and identification of yeast SPO14 as the yeast PLD based on gene sequence homology[38] opened the door for the subsequent cloning of mammalian PLDs.[39-41] The crystal structure of several members of the PLD superfamily and its homologs from cowpea,[42] *Streptomyces sp. Strain PMF*[43-47] and human[48, 49] have been published.

Phosphatidylcholine preferring phospholipase C (PC-PLC) catalyzes the formation of *sn*-1,2-diacylglycerol and choline phosphate. Although the closely related inositol counterpart PI-PLC has been well studied with a number of high-resolution crystal structures from bacterial[50] and mammalian sources[51-53] being made available, the phospholipase C reactive with phosphatidylcholine remains elusive and has not been purified extensively. No PC-PLC isoforms have been cloned from mammalian sources, nor has the mechanism by which the enzyme is involved in the cell response to mitogens and induction of DNA synthesis been fully elucidated. The occurrence of this enzyme in

tissue, however, is expected because an enzymatic activity exists that produces choline phosphate from phosphatidylcholine.

Despite the potential significance of PC-PLCs in eukaryotic signal transduction, cell-free studies on such enzymes are scarce. Only on a few occasions has PC-PLC been moderately purified. Wolf partially purified a 29-kDa neutral PC-PLC by anion exchange, hydroxylapatite, chromatofocusing, and gel filtration chromatography from the cytosolic fraction of canine myocardium.[54] Srivastava isolated an enzyme, composed of two subunits of 69 and 55 kDa, from the 70% ammonium sulfate extract of bull seminal plasma and the enzyme was purified to homogeneity through multiple chromatographies.[55] By use of an antibody raised in New Zealand white rabbit against the PC-PLC from *Bacillus cereus*, a one-step, 3000-fold enrichment of phosphatidylcholine preferring phospholipase was achieved from human monocytic U937 cells.[56] Based on the observed antigenic similarity of mammalian and bacterial PC-PLCs, it was suggested that they might be structurally and evolutionarily related.[56] This enzyme was calcium dependent, most active at neutral pH and inhibited by EGTA. SDS Gel electrophoresis revealed it had a molecular weight of 40 kDa, nearly twice as heavy as *Bacillus cereus* phospholipase C to be discussed later.

1.2.2 Bacteria PC-Preferring Phospholipase C

Thus far, none of the mammalian PC-PLCs have been characterized with respect to primary protein structure. Most studies were in the past devoted to the isolation, cloning and characterization of prokaryotic PC-PLC enzymes.[57] However, PC-PLCs have been detected in a wide variety of bacteria.[57] All of the bacterial PC-PLCs reported to date are single polypeptide proteins, which are found in the culture media. The enzymes have been purified by a variety of techniques: for examples, an affinity

chromatography on agarose-linked egg yolk lipoprotein has been used to purify the *Bacillus cereus* phosphatidylcholine-preferring phospholipase C (PC-PLC)[58] and the *C. perfringens* enzyme.[59-61] *Pseudomonas aeruginosa* phospholipase C was purified by binding to ammonium groups on DEAE-Sephacel followed by eluting with tetradecyltrimethylammonium bromide.[62]

Several of the bacterial phospholipases, such as those sourced from *Clostridium perfringens*,[63-65] *Bacillus cereus*[66] and *Staphylococcus aureuse*,[67] have been characterized by their amino-acid sequence, and the respective DNAs have been cloned. The primary structures have revealed typical signal sequences.[68, 69] Among them, the *C. perfringens* alpha-toxin and *B. cereus* PC-PLC are the most intensively studied and therefore the best characterized.

C. perfringens alpha-toxin PC-PLC is involved in the symptom of gas gangrene and is a potent toxin with hemolytic, lethal, dermonecrotic, vascular permeabilization and platelet-aggregating properties. In contrast, the enzyme from *B. cereus* is nontoxic, non-hemolytic,[70] and does not cause platelet aggregation[71] or hydrolyze sphingomyelin.

PLC from *B. cereus* are widely available from commercial supplies and its purification protocol has been well established. As a result of extensive investigations in the past 25 years, this enzyme has acquired the status of a prototype bacterial PC-PLC. The extracellular *B. cereus* PC-PLC is posttranslationally activated by the removal of 14 *N*-terminal amino acids.[66] This enzyme is believed not to be involved in cell signal transduction, and *in vivo*, the only known function is phosphate retrieval in times when phosphate becomes limited. Antibodies to PLC_{Bc} have shown to cross react with at least some of the mammalian activity as mentioned previously.[56] Several experiments also demonstrated that PLC_{Bc} can mimic mammalian PC-PLC *in vitro*. Levine observed increased arachidonic acid metabolites in rat liver cells (the C-9 cell line), porcine aorta

endothelial cells, bovine aorta smooth muscle cells, bovine aorta endothelial cells, mouse fibroblasts and rat keratinocytes cell culture after treatment with PLC_{Bc}.[72] Johansen[73] reported that constitutive expression of the gene encoding PC-PLC from *B. cereus* led to chronic elevation of the cellular DAG level and oncogenic transformation of NIH 3T3 cells. The expression of the bacterial PC-PLC gene was both necessary and sufficient for induction and maintenance of the transformed phenotype. Moscat observed that the exogenous addition of PC-PLC from *B. cereus* was able to cause mimicking of both a significant portion of the mitogenic response to platelet-derived growth factor (PDGF) in Swiss 3T3 fibroblasts and the constitutive activation of protein kinase C (PKC) in ras- or src-transformed NIH 3T3 cells.[74, 75]

The phosphatidylcholine-preferring PLC_{Bc} is a 28.5-kDa monomeric metalloenzyme containing 245 amino acids and three zinc atoms in its active site. The enzyme processes PC preferentially, relative to PE and PS (ratio of k_{cat} = 10:5:2, k_{cat}/K_m = 9:6:1).[76] Crystallographic[77] and chemical modification[78] studies of *B. cereus* PC-PLC have provided an insight into the molecular architecture of this enzyme and, by extrapolation, the possible tertiary structures of homologous regions in other zinc-binding phospholipase C. The enzyme is quite resistant to denaturation by urea, sodium dodecylsulfate or heat (70 °C for 10 min), probably due to the structural stabilization of the zinc ions.[79]

The three-dimensional crystal structure of *B. cereus* PC-PLC at 1.5Å resolution revealed that the enzyme is composed of ten helices, forming a roughly ellipsoidal structure with approximate dimensions of 40 Å x 30 Å x 20 Å (Figure 1.4).[77, 80] The molecular surface is smooth except for a cleft, which is approximately 8 Å deep and 5 Å wide. Neutral, basic, and acidic amino acid residues are evenly distributed on the molecular surface of PLC_{Bc} except for two regions located close to the active site cleft. In

the first region, amino acid residues form a non-polar hydrophobic surface, while residues in the other region are primarily acidic and in close proximity to the metal ions. The crystal structure confirmed the presence of three zinc ions, all within the cleft, refuting the traditional view of two from atomic absorption analysis. These metal ions appear to play important roles in stabilizing the enzyme since they are coordinated to widely separated segments of the peptide chain. The three zinc ions, one of which is loosely bound, coordinate with amino acid residues from different helices and thus conformationally restrain the tertiary structure. All three zinc icons in the native structure are pentacoordinated: Zn1 is coordinated by two histidines (His69 and His118), two aspartic acids (Asp55 and Asp122) and an active-site water. Zn2 is ligated with two histidines (His128 and His142) and glutamic acid-146 with the remaining two covalent vacancies filled with active site waters, and Zn3 is loosely bound with histidine 14, aspartic acid 122 and the carboxyl and *N*-terminal amino groups from tryptophan 1 and a water molecule. All three zinc ions appeared to have three oxygen and two nitrogen ligands in what could be described as trigonal bipyramidal coordination. Asp 122 forms a carboxylate bridge between Zn1 and Zn3 (Zn-Zn distance 3.3Å), and an active-site water forms a second bridge between these two zinc atoms (Figure 1.5).

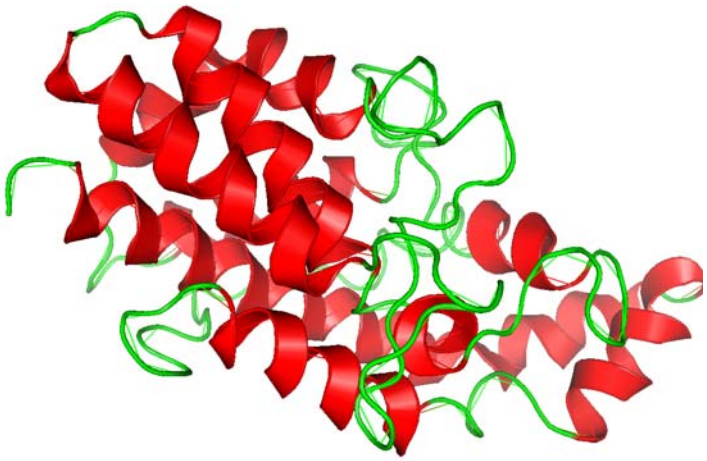


Figure 1.4: An illustrate drawing of PL-PLC from *B. cereus*.

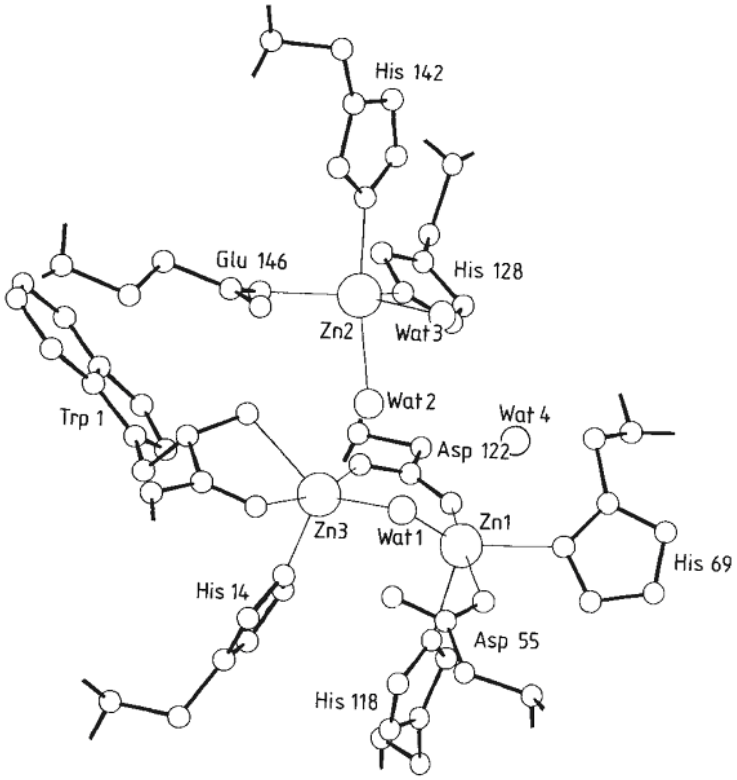


Figure 1.5: The active site of PL-PLC from *Bacillus cereus* showing the coordination between amino acid resides and three zinc ions.[77]

A number of X-ray crystal structures of PLC_{Bc} complexed with several different ligands have also been solved.[80-82] The active site of *B. cereus* PC-PLC was tentatively identified by examining the cocrystal of the enzyme with an inorganic phosphate.[77] This assignment was later confirmed by a structure of PLC_{Bc} complexed with a phosphatidylcholine-derived phosphonate inhibitor.[80] This crystal structure revealed that the phosphonate binds to the active site in a similar fashion to inorganic phosphate. The two non-bridging oxygen atoms of the phosphonate bind to all three zinc ions, displacing two of the coordinating water molecules in the active site of native PLC_{Bc}, one of which bridging Zn1 and Zn3 and the other coordinating to Zn2 in the native structure of the PLC_{Bc}. The major difference is that the other Zn2 ligand water appeared to be gone as well, thus leaving Zn2 tetracoordinated, rather than pentacoordinated as it was in the native structure (Figure 1.6). This tri-metal-center binding pattern resembles that of *E. coli* alkaline phosphatase[83, 84] and P1 nuclease from *Penicillium citrinum*.[85, 86]

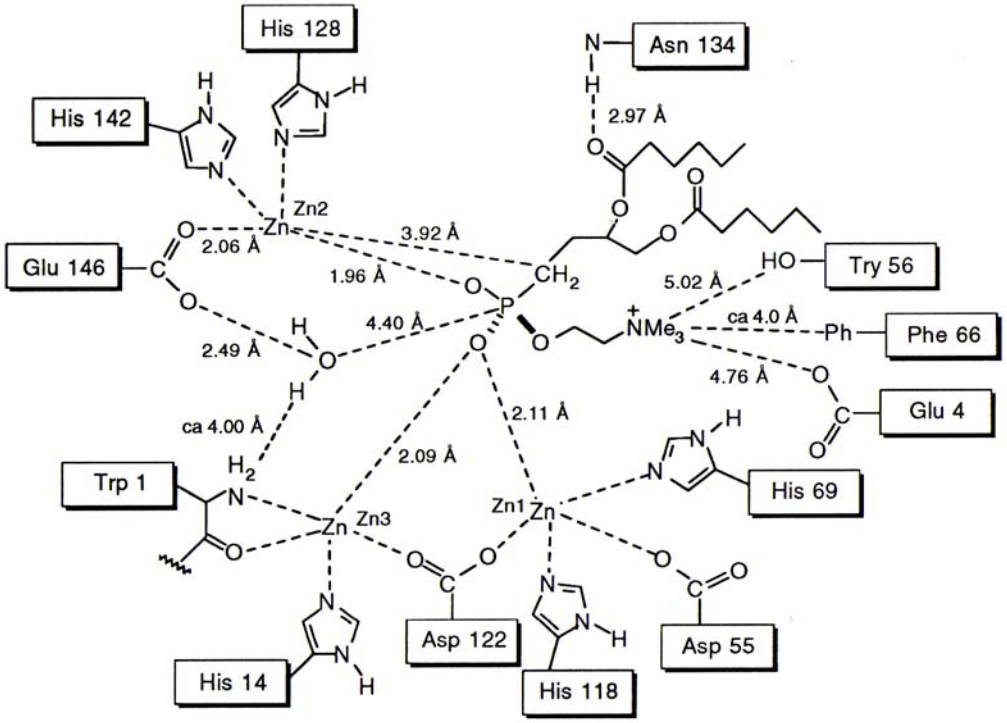


Figure 1.6: Interaction at the active site of PLC_{Bc} complexed with a non-hydrolyzable substrate analog.[80, 87]

E. coli alkaline phosphatase (AP) has two zinc and one magnesium ions at the active site (Figure 1.7). Extensive investigations into the catalytic mechanism of AP resulted in well-accepted kinetic scheme for the enzymatic hydrolysis/transphosphorylation of phosphate monoesters, establishing this particular bacterial enzyme as a model for other phosphatases that use metal ions in phosphate ester hydrolysis and transphosphorylation reactions.[88, 89] It was accepted that the nucleophilic Ser 102 is transiently phosphorylated giving a covalent phosphorylated-enzyme intermediate, which is then hydrolyzed to release the inorganic phosphate. Isotope labeling with a chiral (^{16}O, ^{17}O, ^{18}O) phosphoryl diester conformed that the hydrolytic

reaction proceeded with overall retention of configuration at the phosphorous atom,[90] consistent with the double displacement mechanism *via* in-line attack and the formation of a covalent phosphorylated enzyme intermediate. The detailed mechanism invokes a rather classic two metal ion catalysis with a zinc ion activating the nucleophilic hydroxyl and the other stabilizing the leaving alkoxide. Both zinc ions chelate to the non-bridging oxygen on the phosphorus atom. The magnesium ion appeared to be required to structurally stabilize the enzyme in its catalytically most active form and is not essential for reactivity. The enzyme with three zinc ions is still active, and elimination of the functional link between Mg and Zn2 in the D51N mutant enzyme resulted in the vacancy of the Mg site with a mere 20-40 fold loss of enzymatic activity being observed.[89]

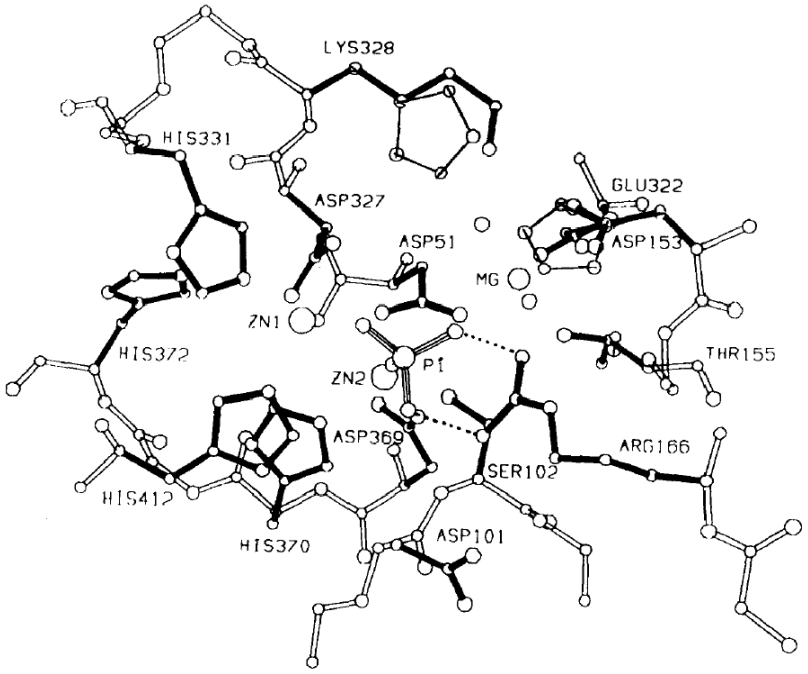


Figure 1.7: Active site of the *E. coli* alkaline phosphatase. Some residues and water molecules are omitted for clarity. In the mammalian sequences all the residues involved in the metal binding are conserved except for Thr155, which is replaced by serine in some cases. Others in this region are also conserved as well except for a few.[83]

P1 nuclease from *Penicillium citrinum* is also a phosphodiesterase, cleaving the bond between 3'-hydroxyl and 5'-phosphoryl group of the adjacent nucleoside in the nucleic acid, and at the same time, acting as a phosphomonoesterase to remove the 3'-terminal phosphate group.[91] In its active form, P1 nuclease contains three zinc ions per molecule.[92] Superimposition of the three dimensional structures *Penicillium citrinum* P1 nuclease and *B. cereus* phospholipase C revealed a striking similarity despite the fact that the two enzymes share only 18% of the sequence identity in the superimposed segment. Eight of the nine α-helices in PLC_{Bc} appear to correspond to α-helical segments in P1 nuclease. The zinc-coordination pattern is essentially the same in both enzymes, including the presence of three water ligands. The only difference in zinc-coordination is that an aspartic acid at position 153 in P1 nuclease coordinated to Zn2, which comes out to be a glutamic acid in PLC_{Bc}. The stunning similarity in three-dimensional structure and almost identical disposition and coordination of the three zinc ions has led to the probably false assumption that P1 and PLC_{Bc} may be evolutionary related and share similar hydrolytic mechanism.[86, 93] The crystal structure of P1 with a non-hydrolyzable *R*-diastereomer of monothiophosphorylated oligonucleotides, solved at 2.8Å, revealed significant differences in substrate binding. The relatively inaccessible dinuclear Zn ions (Zn1 and Zn3) don not make direct contact with the ligand, and the more exposed Zn2 is the only zinc ion that bound to the phosphate group. Arginine 48, where no analogous amino acid residue found in PLC_{Bc}, is in close proximity to the phosphate, probably stabilizing the negative charge in the pentacovalent transition state with its positively charged guanidinium group.

Several possible hydrolytic mechanisms have been envisaged,[86, 94, 95] and all of these involve nucleophilic attack by a zinc activated water molecule and stabilization of the pentacovalent transition state by Arg48 without the involvement of a covalent

phosphorylated enzyme intermediate. However each mechanism assigns different roles to the three zinc ions and the firmly bound water molecules seen in the P1 crystal structure. The overall stereochemistry at phosphorous center hence is inverted, compatible with experimental discovery using isotope labeled thiophosphorylated substrate. The reaction mechanism was investigated using single-stranded dithiophosphorylated oligonucleotides as substrate analogs in Sucks' group. Based on the 1.8 Å structure, he suggested that the water molecule bridging Zn1 and Zn3 acted as the nucleophile for an in-line attack on the phosphodiester P-O bond. Asp 45, which also serves as a ligand of Zn1, helps to orient the hydroxide for attack.[96] The resulting pentacoordinate intermediate with the additional negative charge was stabilized by Arg48 guanidinium with the incoming hydroxide ion and the leaving O3 occupying apical positions. The more exposed Zn2, as a Lewis acid, plays a dual role in activating the phosphate and stabilizing the leaving O3-alkoxide (Figure 1.8). Thus all three zinc ions are essential for catalysis.

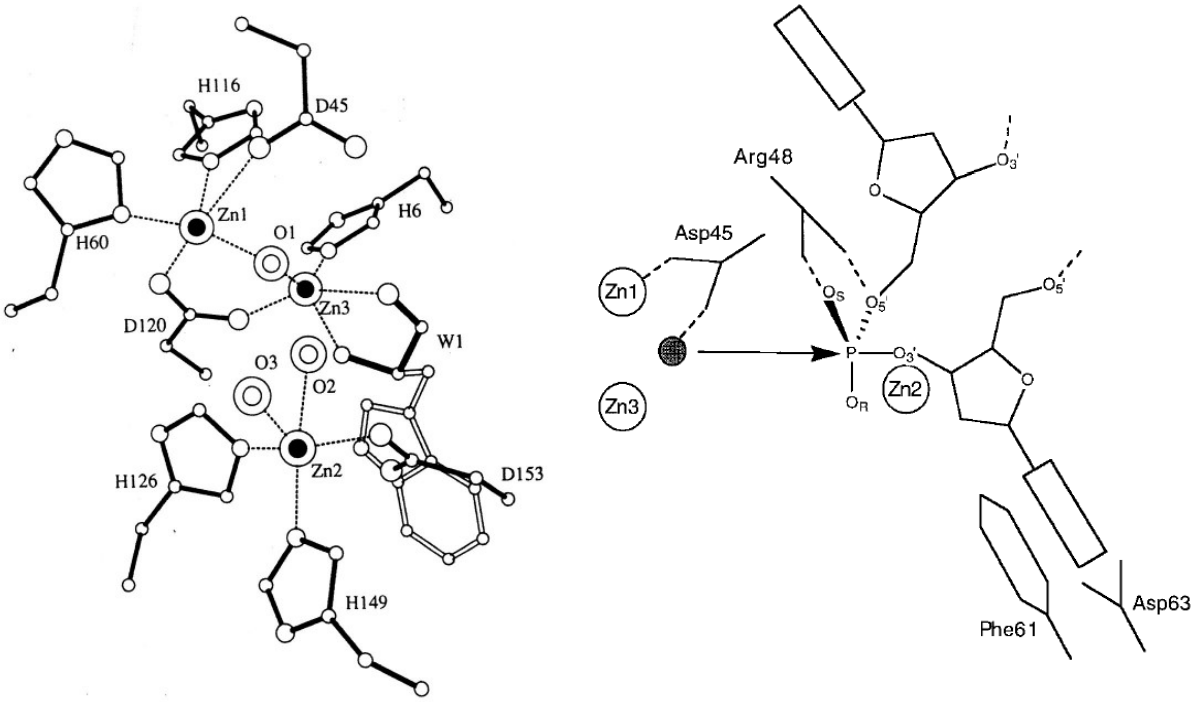


Figure 1.8: On the left: Zn coordination in P1 nuclease. Carbon, nitrogen and oxygen atoms are represented by open spheres with radius depending on atom type. Zn ions (Zn1, Zn2 and Zn3) and water ligands (O1, O2 and O3) are depicted by two concentric sphere. Dashed line indicate Zn-ligand interaction.[86] On the right: Proposed catalytic mechanism of P1 nuclease based on a crystal structure with a substrate analog. The hydroxide ion (shaded sphere) bridging Zn1 and Zn3 and Zn2 is stabilizing the leaving O38-oxyanion.[96]

One of the noticeable features in the PLC_{Bc}-inhibitor complex is that the choline head group adopts an unusual orientation in which it is folded back over the diacylglycerol part of the inhibitor and is almost parallel with the two acyl side chains. Three amino acid residues Glu4, Tyr56, and Phe66 comprise the site to which the positively charged choline head group binds (Figure 1.9).[80] The carboxyl group on the side chain of Glu4 is 3.9Å away, perhaps stabilizing the choline positive charge *via* electrostatic interaction. The center of the aromatic rings of Tyr56 and Phe66 are 4.7Å and 4.2Å from the positively charged methyl group, respectively. They are positioned so that one π-face of the respective aromatic rings, which have partial negative character due to the quadrupole moment, is coordinated with choline trimethylammonium cation to stabilize the opposite charged, probably through cation-π interaction.[97-100] Those interactions around the positively charged trimethylammonium ion are important for the substrate binding and catalysis as a phosphatidylcholine analogue with the trimethylammonium moiety replaced by an isosteric *tert*-butyl group was found to undergo hydrolysis 1000 times less efficiently by PLC_{Bc}.[101]

The other two natural substrates of PLC_{Bc}, phosphatidylethanolamine (PE) and phosphatidylcholine (PS), probably also bind to the active site in a similar fashion. It seems reasonable to assume that Glu4, Tyr56, and Phe66 contribute differently in stabilizing/destabilizing the charged head groups of the phospholipids, whether it is the

positively charged trimethylammonium/ammonium ion or the negatively charged carboxylate. These amino acid residues could therefore play roles in dictating the substrate specificity of PLC_{Bc}.

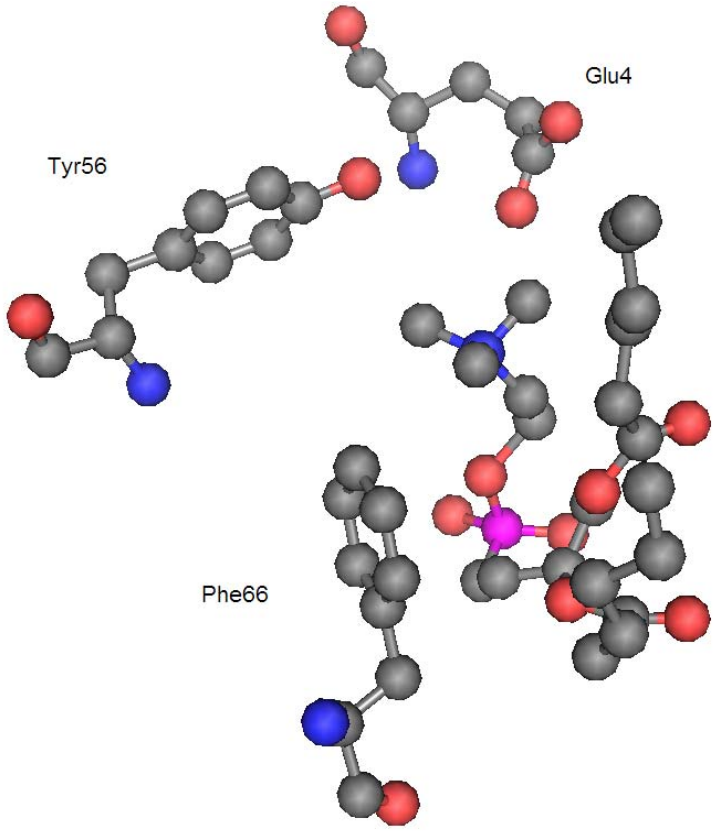


Figure 1.9: View of Glu4, Tyr56, and Phe66 interacting with a substrate analogue inhibitor; the distances between the methyl groups of the ammonium group of the choline moiety and the center of the aromatic rings of Tyr56 and Phe66 and the carboxyl group of Glu4 are shown.

Less is known about the interaction between the amino acid residue of PLC_{Bc} and the two acyl side chains. No clear-cut interactions could be identified except a hydrogen bonding between *sn*2-carbonyl and asparagine 134. Both side chains of the diacylglycerol moiety are somewhat disordered with the *sn*-1 acyl chain bring more loosely bound. The

thermal motions increase along the aliphatic tail. The lacking of any obvious interactions come with a surprise since phosphorylated serine, ethanolamine and choline themselves do not bind to PLC_{Bc} and phospholipids with longer side chains are generally more reactive substrates.

Base on evidence from X-ray crystallography and kinetic analysis, and in analogy to other phosphodiesterases,[102] it was proposed that PLC_{Bc} hydrolyzes a phosphodiester by activating an active site water molecule for in-line attack on the phosphodiester. Collapse of the resultant pentacoordinated intermediate then provides a diacylglycerol and a phosphorylated head group with inversion of configuration at phosphorus atom.

A series of studies were conducted to understand the mechanistic aspects of the PLC_{Bc} catalyzed hydrolysis of phospholipids in the Martin group.[103] The absence of the solvent viscosity effects suggested that an internal, chemical step was the rate-determining step for hydrolytic reaction of water-soluble phospholipids, refuting the proposal that release of DAG is rate determining.[103, 104] Solvent isotope effect and proton inventory studies indicated a single proton transfer; either the deprotonation of the nucleophilic water or the protonation of the leaving cholinephosphate, is involved in the rate-limiting step.[103] Mutagenesis studies provided evidence to support Asp55 rather than Glu4 to be the general base that activated the water for nucleophilic attack on the phosphodiester, and mutations at this position generate enzymes having similar structures to that of the wild type but whose catalytic activities are reduced by 10^4-10^6 fold.[105, 106]

1.3 CHEMICAL SYNTHESIS OF PHOSPHOLIPIDS AND THEIR ANALOGS

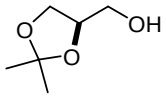
The phospholipids to be discussed in this and upcoming chapters are generally phosphomonoester and phosphodiesters. The key issues in the chemical synthesis of phospholipids are the generation and preservation of the stereocenter at *sn*-2 position,

introduction of the fatty acid side chains in a site-specific manner and the incorporation of phosphatidyl diester functionality.[107-110]

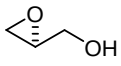
1.3.1 A Brief Overview of Phosphorylation

The key issue in the syntheses of phospholipid is the generation of a well-defined stereocenter at the glycerol 2-position followed by introduction of the fatty acid substituents in a regiospecific manner that preserves the stereochemical integrity of the central carbon.[111] As a result, most stereospecific synthetic approaches that have been developed require multiple protecting group manipulations, which is particularly true for those methods aimed at preparing phospholipids with different acyl groups.

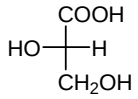
The *sn*-2 stereocenters are generally derived from chiral pools. Most of the approaches developed to date still follow the synthetic scheme devised by Baer in his landmark synthesis of 1,2-distearoyl-*sn*-glycero-3-phosphorylholine in 1950,[112] which involved derivatization and protecting group manipulations of an orthogonally protected glycerol derivative.[87, 107, 112-116] The glycerol derivatives were often obtained from 1,2-isopropylidene-*sn*-glycerol (**1.1**), a currently commercially available material that is also readily available from D-mannitol.[117] They can also be prepared through functionalization of other commercially available materials, such as L-glyceric acid (**1.3**).[111]



1.1



1.2

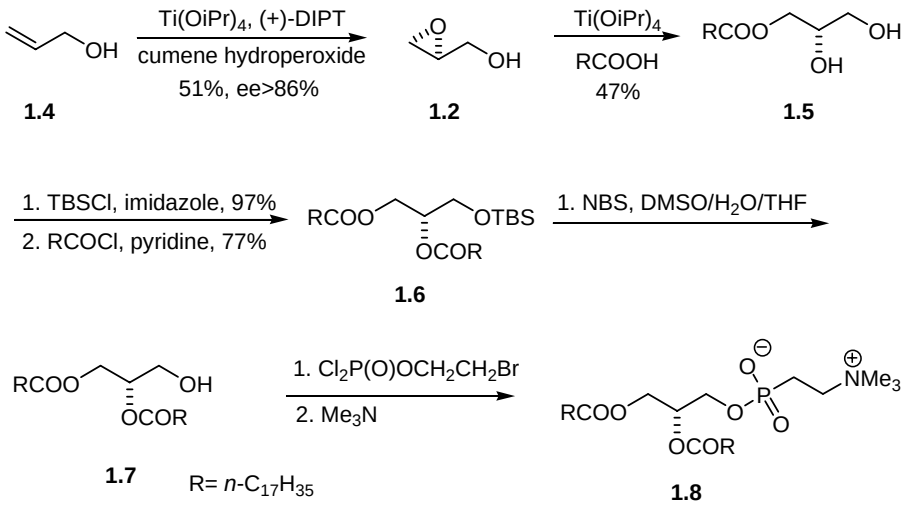


1.3

Figure 1.10: Commonly used chiral starting materials for phospholipids synthesis

Methods employing derivatives of enantiomerically pure *S*-glycidol (**1.2),** which became available from stereospecific epoxidation of allylic alcohols, for preparing optically active glycerophospholipids and analogue have recently been published.[118-120] In Burgos' approach, the titanium-assisted nucleophilic opening of *S*-glycidol (**1.2**), which was prepared *via* Sharpless asymmetric epoxidation of allylic alcohol, with stearic acid gave (*S*)-(+)-1-stearoyl-*sn*-glycerol (**1.5**).[118] Selective silylation of the primary alcohol in **1.5** with *tert*-butyldimethylsily chloride followed by esterification of the resultant secondary alcohol with stearoyl chloride and removal of the silyl protecting group afforded distearoyl-*sn*-glycerol **1.7**.[118] Exposure of **1.7** to 2-bromoethoxy-phosphonic dichloride followed by displacement of the bromide with trimethylamine according to literature precedence would provide (*R*)-1,2-distearoyl-*sn*-glycero-3-phosphorylcholine **1.8** (Scheme 1.1).

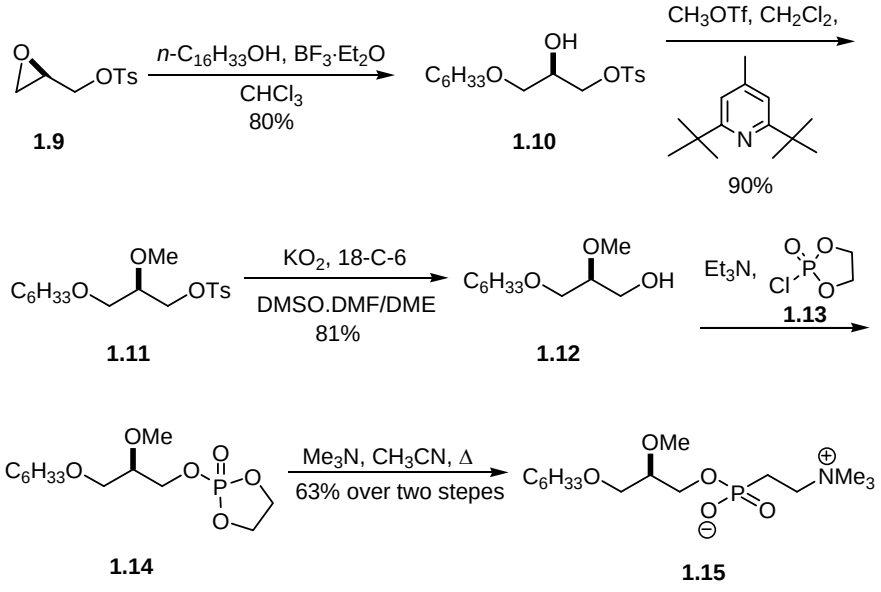
Scheme 1.1



Guivisdalsky prepared ether-linked phospholipids (alkyl phospholipids) from *R*-glycidyl tosylate (**1.9**) and *R*-glycidyl *tert*-butyldiphenylsilyl ether, which have the same

absolute stereochemistry at C2 as *S*-glycidol (**1.2**).[120] Regio- and stereospecific nucleophilic opening of *R*-glycidyl tosylate with 1-hexadecanol in the presence of $BF_3{\cdot}Et_2O$ as catalyst and subsequent *O*-methylation under mild conditions afforded dialkylglycerol **1.11** without concomitant formation of epoxide. Treatment of **1.11** with potassium superoxide in the presence of 18-crown-6 led to the direct displacement of tosylate to form alcohol **1.12**. The phosphocholine moiety was introduced by reaction of **1.12** with 2-chloro-2-oxo-l,3,2-dioxaphospholane (**1.13**), followed by heating the cyclic dioxaphospholane intermediate **1.14** with trimethylamine, affording phosphorylcholine **1.15** in good yield (Scheme 1.2).[121] Alkyl phospholipids with different head groups could also accessed by opening the dioxaphospholane with a variety of nucleophiles in the presence of trimethylsilyl triflate.[122] The method starting from *S*-glycidol or its derivatives has found widespread application to the synthesis of ether lipids, yet it turned out to be less applicable to the syntheses of diacyl phospholipids.[109]

Scheme 1.2



A second issue in the phospholipid synthesis is the introduction of the phosphodiester functionality. Thanks to the development of new methods originally designed for the phosphorylation of nucleic acids, peptides, carbohydrates and steroids, numerous reagents and approaches are now available to address the issue of phosphorylation of protected glycerol derivatives. Most of the approaches involved either activation of the phosphorylating reagent, the alcohol or both. Some commonly used phosphorylating reagents are dichlorophosphate **1.16** (Scheme 1.1), chlorophosphate **1.17** (acid chloride) (Scheme 1.3), pyrophosphate **1.18** (acid anhydride), phospho-1,2,4-triazolide **1.19**, phosphobenzotriazole **1.20** and phsophoimidazole (Figure 1.11). Phosphoric acid can also be used in conjunction with trichloroacetonitrile, arylsulfonyl chloride (2,4,6-triispropylbenzene sulphonyl chloride) or carbodiimide (Scheme 1.4). Alcohol can be activated as its corresponding epoxide or alkyl halide, and phosphorylation is achieved by nucleophilic substitution with a metal phosphonate. The silver phosphonate is commonly used to activate the phosphate concurrently (Scheme 1.3). Because of the close resemblance to ester syntheses, the details of phosphorylation using phosphate chemistry will not be discussed. Instead, a brief discussion of the chemical synthesis of phospholipids *via* P (III) derivatives will be presented.

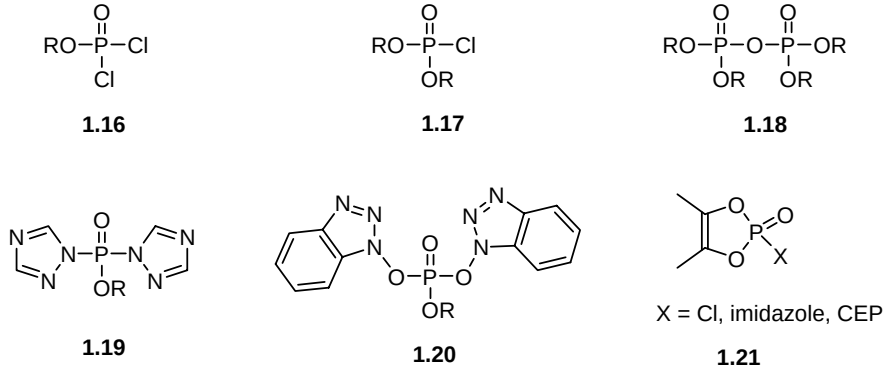
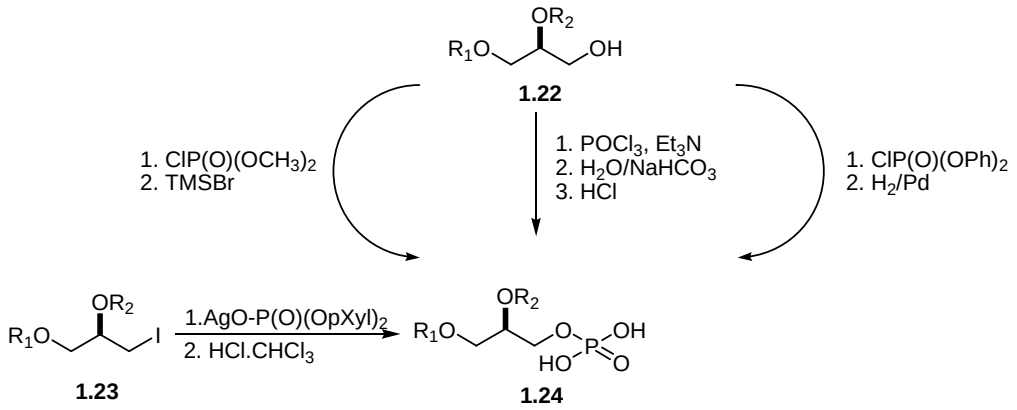
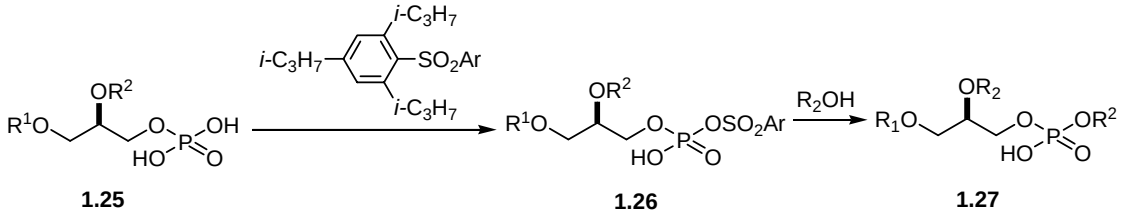


Figure 1.11: Commonly used P(V) phosphorylating reagents.

Scheme 1.3



Scheme 1.4

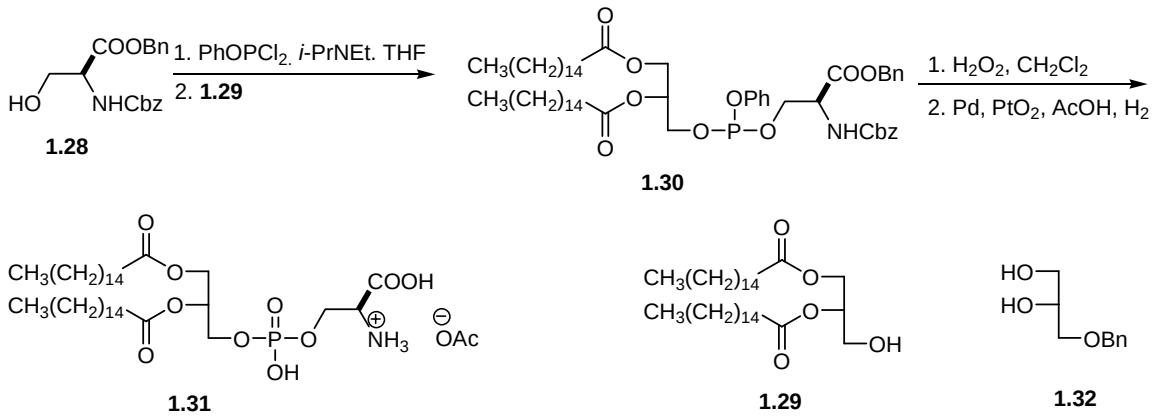


Tri-coordinate phosphorus compounds containing suitable leaving groups are indispensable phosphorylation reagents for the syntheses of phospholipids and their analogues. Nucleophilic displacements on the electrophilic phosphorus and subsequent oxidation of the resultant P(III) compound is one of the most important routes to biophosphates. P (III) compounds can also behave as nucleophiles due to the presence of a free electron pair, reacting with a wide range of electrophiles to give P(IV) compounds, which have been converted into P(V) compounds. This second advantageous property allows conversion of P (III) compounds into biophosphates and their important analogues such as phosphorothioates and phosphonates.[123]

Chlorophosphite and dichlorophosphite are among the first being used in the synthesis of phospholipids. Martin has developed a procedure of coupling simple

alcohols with two common polar head groups (L-serine and ethanolamine).[124] For example, phosphatidylserine **1.31** was prepared in six steps (45% overall yield) from diol **1.32** via a sequence that featured the phosphite coupling of diacylglycerol **1.29** with benzyl carbobenzyloxyserine (Scheme 1.5).

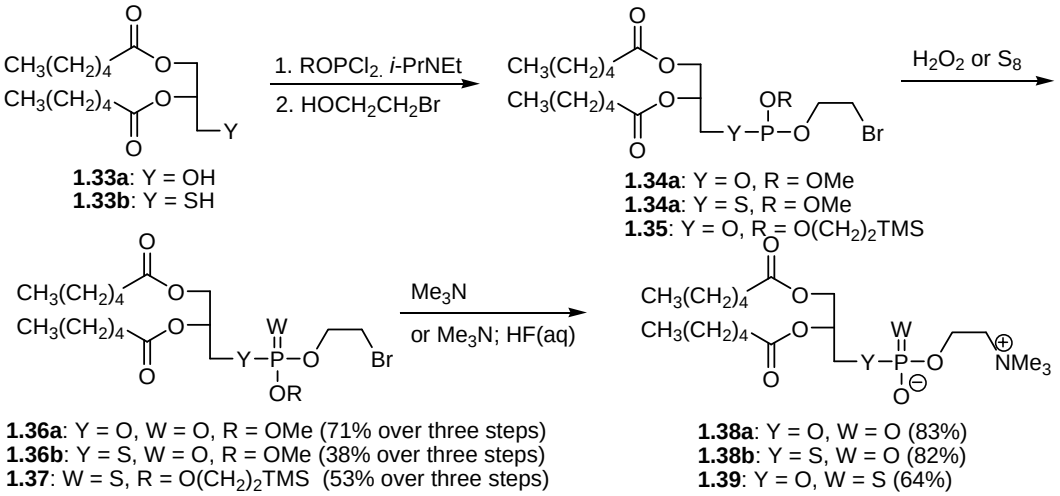
Scheme 1.5



Phosphatidylscholine could prepared similarly though the corresponding bromoethanol analog such as **1.36a**.[124] Phosphite coupling of the diacylglycerol **1.33a** and bromoethanol using methyl dichlorophosphite delivered an intermediate phosphite triester **1.34a** that was oxidized with hydrogen peroxide to furnish the phosphate triester **1.36a** (Scheme 1.6). Deprotection of the *O*-methyl group in **1.36a** by trimethylamine with concomitant nucleophilic displacement of the bromide gave the phosphatidylcholine analogue **1.38a**. The phosphorothioate analogue **1.39** could be readily prepared from the triester **1.37.** Triester **1.37** was in turn prepared *via* the coupling of the diacylglycerol **1.33** with 2-bromoethanol using 2-(trimethylsilyl)ethyl dichlorophosphite followed by sulfurization of the intermediate phosphite triester **1.35**. Reaction of **1.37** with trimethylamine and subsequent deprotection using dilute aqueous hydrofluoric acid gave

choline phosphorothioate **1.39**. Martin also demonstrated that thiophospholipid, such as **1.38b**, could be synthesized likewise.[87] The diacylglyceryl thiol **1.33b** was coupled with bromoethanol using methyl dichlorophosphite, and the thiophosphite triester **1.34b** thus obtained was oxidized to give the thiophosphate triester **1.36b**. Reacting of **1.36b** with trimethylamine provided the choline thiophosphate **1.38b**.

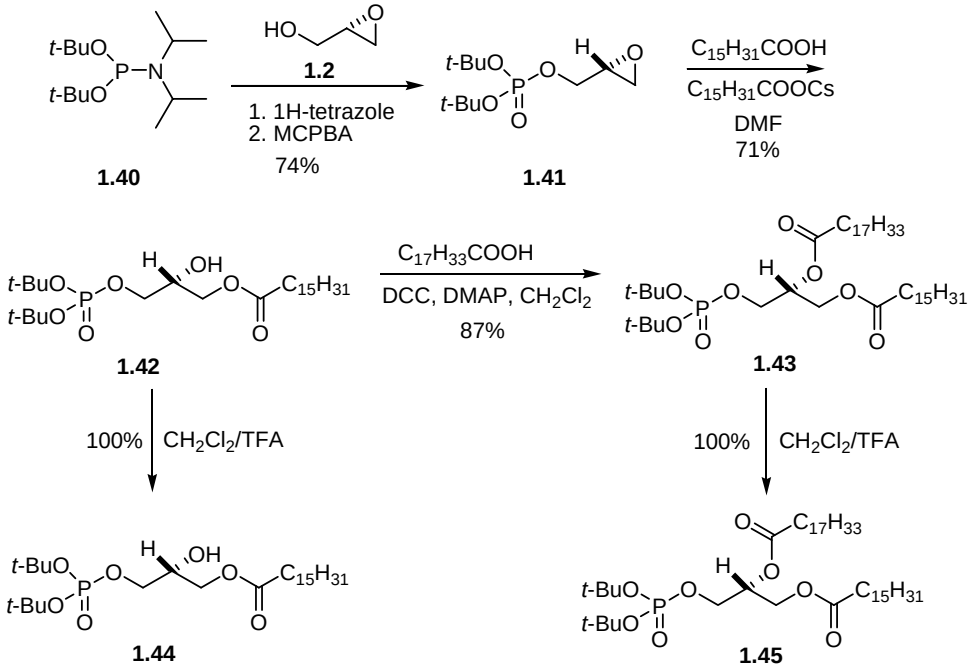
Scheme 1.6



Phosphoroamidites are nowadays the most commonly used phosphorylating reagents for the synthesis of various biomolecules. However, they do not react readily with alcohols unless a proper activator, such as tetrazole, is employed.[123, 125] Konradsson recently developed a route to enantiopure phospholipids utilizing a phosphoroamidite coupling. Thus lysophosphatidic acid **1.44** and phosphatidic acid **1.45** were obtained in good overall yields in only three and four steps from *S*-glycidol (**1.2)**.[126] The oxidative phosphorylation of (*S*)-glycidol **(1.2**) with dialkyl phosphoramidite **1.40** furnished phosphorylglycidol **1.41** in 74% yield (Scheme 1.7). Regioselective opening of the epoxide with cesium palmitate afforded the monoester **1.42,** which underwent further

esterification with oleic acid to produce the mixed 1,2-diacyl phosphatidic acid di-*tert*-butyl ester **1.43** in 87% yield. Deprotection of **1.42** and **1.43** with TFA in CH_2Cl_2 produced the lysophosphatidic acid **1.44** and the mixed 1,2-diacyl phosphatidic acid **1.45** in quantitative yields. Coupling of phosphatidic acid **1.45** with choline- and ethanolamine derivatives would lead to phosphatidylcholine and phosphatidylethanolamine. This approach allows both natural and unnatural enantiomers of phospholipids, and analogues thereof, to be obtained in high optical purity from commercially available (*S*)- or (*R*)-glycidol. If an alcohol is used to open the epoxide, then *sn*-1 ethereal analogs of phospholipids could be accessed.

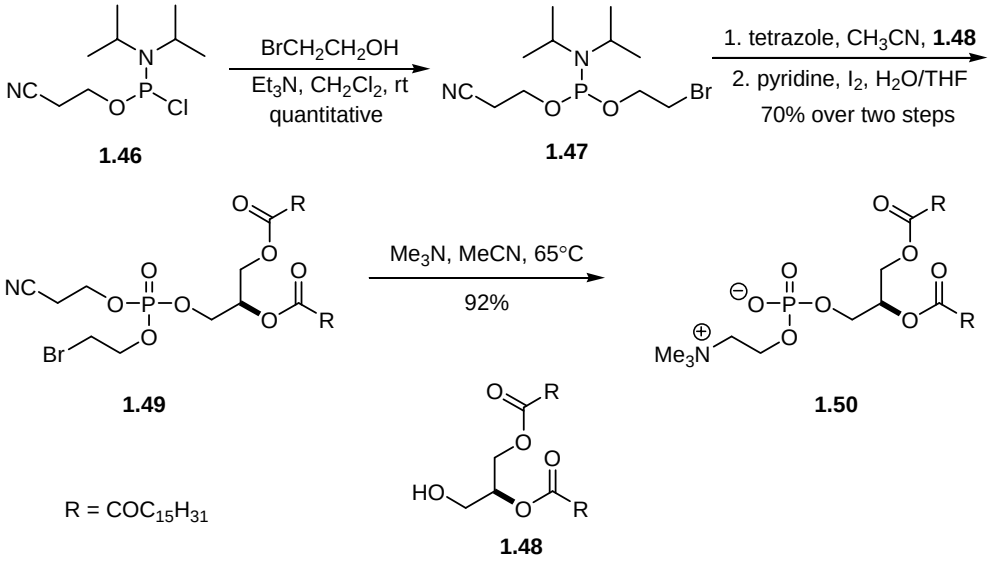
Scheme 1.7



Hebert and Just used an inverse phosphite triester approach in their synthesis of phospholipids (Scheme 1.8).[127] Chlorophosphinite **1.46** was condensed with bromoethanol to give dialkylphosphoramidite **1.47**, which was reacted with

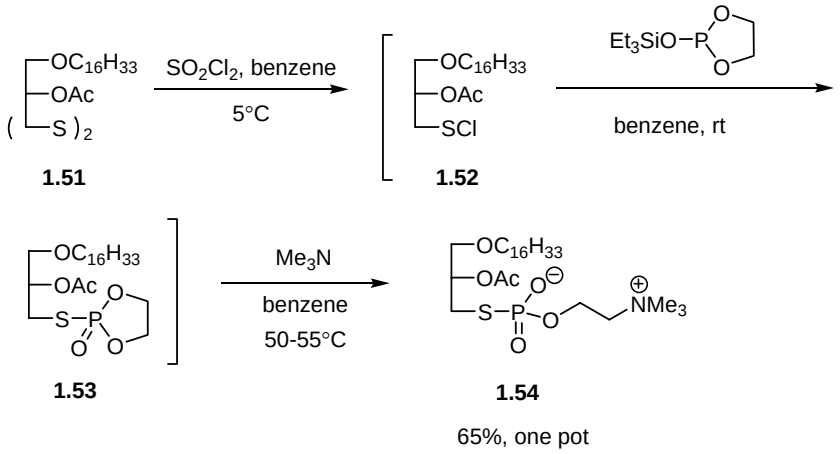
diacylglycerol **1.48** followed by oxidation of the intermediate phosphite *in situ* with iodine affording phosphotriester **1.49**. Treating phosphotriester **1.49** with trimethylamine allowed introduction of the trimethylammonium group and simultaneous removal of the cyanoethyl group, thus producing the zwitterionic phosphatidylcholine **1.50** in 92% yield.

Scheme 1.8



Mlotkowska described the one-pot synthesis of rac-*S*-(2-acetoxy-3-hexadecyloxypropyl)-thiophospholine using an Arbuzov type reaction.[128] The disulfide **1.51** derived from a thioglycerol was chlorinated with sulfuryl chloride to give the corresponding sulfenyl chloride **1.52**. Phosphorylation of **1.52** with triethylsilyloxy-1,3,2-dioxaphospholane afforded thiophospholane **1.53**. The crude thiophosphate **1.53** was then converted into the desired thiophosphocholine by treatment with trimethylamine (Scheme 1.9). This one-pot three-step protocol afforded thiophosphocholine **1.54** in 65% overall yield.

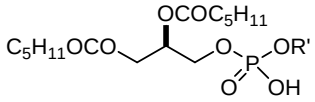
Scheme 1.9



1.3.2 Prior Work From Our Group

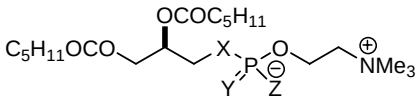
1.3.2.1 Substrate-Based Non-Hydrolyzable Analogs

In the course of the program directed toward elucidating the mechanism of hydrolysis of the phosphodiester bond of phospholipids by enzymes of the phospholipase C class, a number of phospholipids analogues (X = NH, NBn, S, CH_2, and CF_2) of the general type **1.38** were prepared and examined to establish a pattern of structure-activity relationships and to identify those compounds that might be inhibitors of PLC_{Bc} (Figure 1.12). The design of these inhibitors followed the simple principle that altering functional group involved in the transformation, namely the scissile P-O bond of the phosphodiester linkage, to give compounds that were no longer hydrolytically labile. The rest of the structure was then modified to improve the lipophilic interaction, hydrogen bonding and electrostatic interaction in some cases.



1.55

1.55a, **1.38a**: R = choline
1.55b: R = ethanolamine
1.55c: R = L-serine

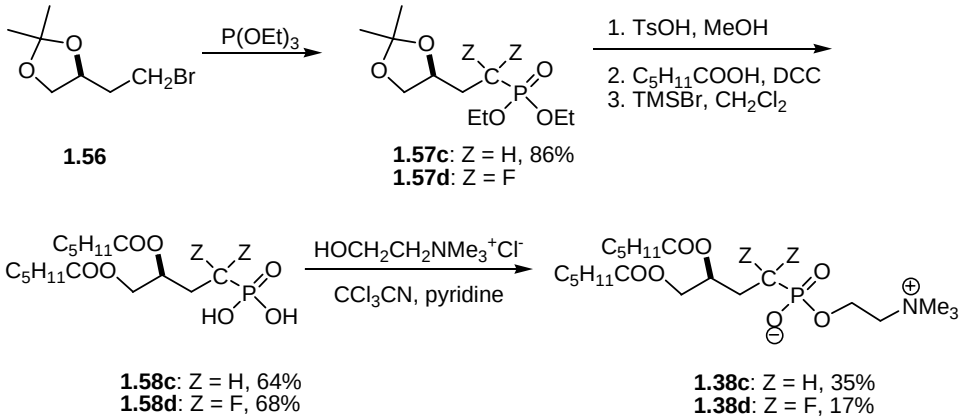


1.38a: X = Y = Z = O
1.38b: X = S, Y = Z = O
1.38c: X = CH_2, Y = Z = O
1.38d: X = CF_2, Y = Z = O
1.38e: X = O, Y = S, Z = O, S_p
1.38f: X = O, Y = O, Z = S, R_p
1.38g: X = O, Y = S, Z = S
1.38h: X = O, Y = S, Z = S, C5 analog of **1.56g**
1.38i: X = NBn, Y = Z = O

Figure 1.12: Substrate-based phospholipase analogs

The phosphonate **1.38c** was prepared by the Arbuzov reaction of the known bromide **1.56** with triethyl phosphite. Cleavage of the acetonide moiety followed by diacylation of the intermediate diol and subsequent hydrolysis of the phosphonate diester provided an intermediate phosphonic acid **1.58c**, which was coupled with choline chloride in the presence of trichloroacetonitrile/pyridine to furnish the phosphatidylcholine analog **1.38c**. Application of a similar sequence of reactions starting with the difluorophosphonate **1.57d**[129], lead to the difluoro analogue **1.38d** (Scheme 1.10).[87]

Scheme 1.10



1.56

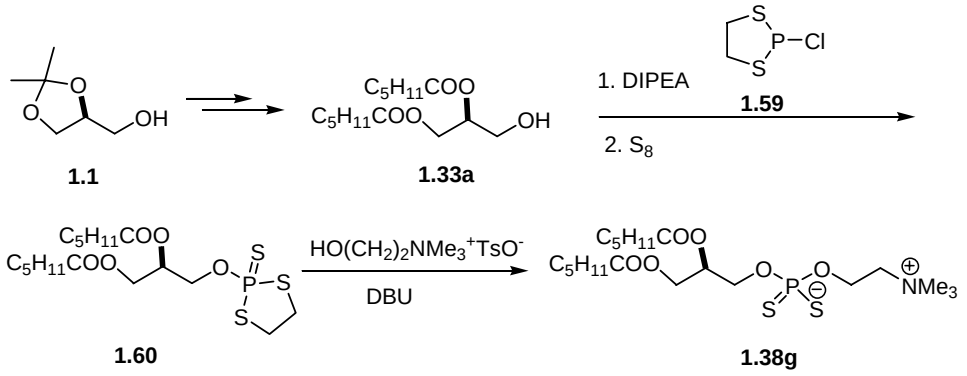
1.57c: Z = H, 86%
1.57d: Z = F

1.58c: Z = H, 64%
1.58d: Z = F, 68%

1.38c: Z = H, 35%
1.38d: Z = F, 17%

Dithiophosphate analog **1.38g** was prepared from 1,2-isopropylidene-*sn*-glycerol (**1.1**). Phosphorylation of **1.33a** with 2-chloro-1,3,2-dithiaphospholane (**1.59**) followed by treating the intermediate phosphite with sulfur provided the 2-alkoxy-2-thio-1,3,2-thiaphospholane **1.60**, which upon exposure to choline tosylate in the presence of DBU delivered **1.38g** (Scheme 1.11).[87, 130]

Scheme 1.11



The phosphoramide analog **1.38i** was also prepared from 1,2-isopropylidene-s*n*-glycerol (**1.1**). Thus, the enantiomerically pure **1.1** was first converted into the *N*-benzyl glycerol **1.61** *via* its tosylate. Phosphite coupling of **1.61** with 2-bromoethanol using methyl dichlorophosphite followed by oxidation of the intermediate phosphite with hydrogen peroxide afforded **1.62** in 78% yield. Subsequent removal of the isopropylidene group and acylation of the intermediate diol with *n*-hexanoic acid in the presence of DCC/DMAP followed by treatment with trimethylamine delivered the phosphatidylcholine analog **1.38i** (Scheme 1.12).[124]

Scheme 1.12

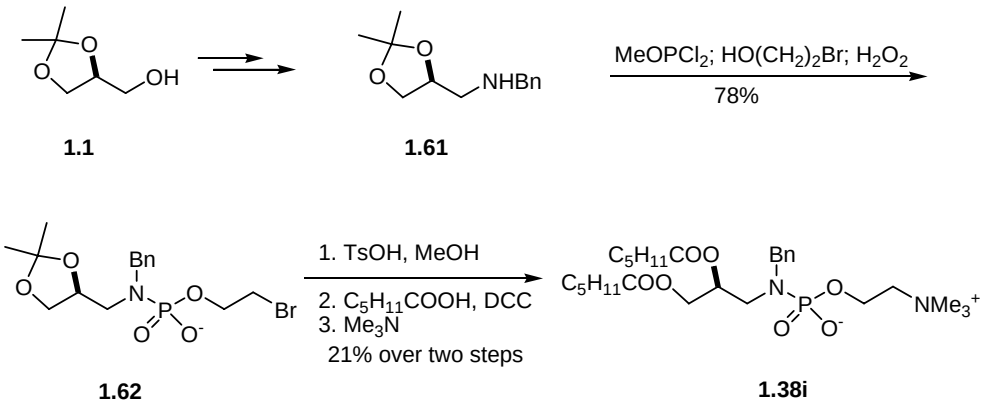


Table 1.1: Results of Kinetic and Solubility Studies on Phospholipid Analogues

Analogue	K_i (mM)	K_M (mM)	Inhibition
1.38a		0.39±0.02	
1.38e: Y = S		0.015±0.001	
1.38i: X =NBn	0.015		competitive
1.38b: X =S	0.007		competitive
1.38c: X =CH_2	1.15		competitive
1.38d: X =CF_2	2.68		competitive
1.38f: Z = S	0.013		competitive
1.38g: Y = Z = S	0.010		competitive

The phosphatidylcholine analogues **1.38a-g** were found to inhibit PLC_{Bc}, and were determined to be competitive inhibitors by examination of the corresponding Lineweaver-Burk plots at different inhibitor concentrations.[87] The thiophospholipid analogues **1.38b**, **1.38f** and **1.38g**, where sulfur replaced one or more phosphate oxygens, were highly effective inhibitors of PLC_{Bc}, with all three being approximately equipotent. Interestingly, thiophosphate **1.38e**, which has the *S* configuration at phosphorus, is a substrate that must bind to the active site with the sulfur atom serving as a ligand for the two structural and cocatalytic zinc ions Znl and Zn3. On the other hand, **1.38f**, which has

the *R* configuration at phosphorus, is an inhibitor that must bind so that the sulfur atom is coordinated to the zinc ion (Zn2) that seems to be involved in catalysis (Table 1.1). Thus, binding of a sulfur ligand to the catalytic zinc ion critically affects the ability of the enzyme to hydrolyze the phosphodiester linkage.

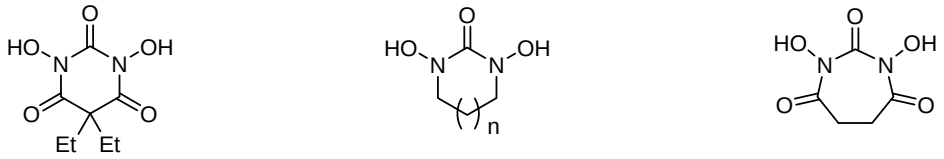
1.3.2.2 Cyclic Zinc-binding N, N'-Dihydroxyurea Inhibitors

As described in the prior sections, most of the inhibitors of PLC_{Bc} synthesized in the Martin group are substrate analogs in which the bridging and/or the non-bridging oxygens have been replaced.[87, 124] However, a large number of non-substrate based inhibitors of PLC_{Bc} have been known. Monovalent anions, such as HCO_3^-, Br^-, Cl^-, NO_3^-, CNO^- and I^-, and Tris were found to be weak inhibitors of PLC_{Bc}.[131] The most notable non-substrate analogue is the xanthate derivative D609 **1.68** (K_i = 15 μM), a potent competitive inhibitor.[132, 133]

The PC-PLC_{Bc} active site contains three zinc ions. The two structural and cocatalytic zinc ions Znl and Zn3 define a dinuclear center bridged by the carboxyl group of Asp122 and a water molecule, with an internuclear distance of 3.5 Å. Thus bidentate ligands that would bind to this bimetallic site could potentially be inhibitors.[134, 135] Hydroxamic acids are well-known stable zinc chelators, and are analogs of the less stable 2,7-dihydroxytropolone (**1.63**), which we have found to be a potent inhibitor of PLC_{Bc} at pH 7.3. It was proposed that the polyhydroxy tropolone inhibits the enzymes by chelating to both metal ions at the active site (Figure 1.13)[134, 136] Hence, a series of hydroxamic acid analogues of **1.63** were prepared and examined as inhibitors of PLC_{Bc}.

1.63

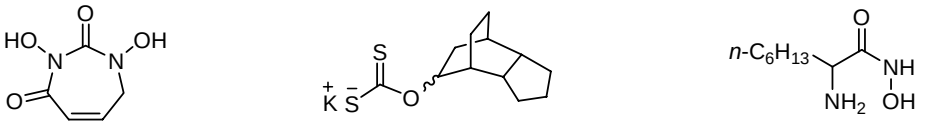
Figure 1.13: Proposed mode of chelation of the two metal ions by the dianion of 7-hydroxytropolone



1.64

1.65a: n = 0
1.65b: n = 1
1.65c: n = 2

1.66



1.67 **1.68** **1.69**

Figure 1.14: Non-substrate-based PLC_{Bc} inhibitors

Dr. Follows prepared compounds **1.65a-c** and **1.66** in a straightforward fashion that relied on the dialkylating of an *O*-protected hydroxylurea **1.70** with 1,ω-dibromoalkane such as **1.71** or succinyl chloride **1.72** (Scheme 1.13). The unsaturated dicarbonyl hydroxamic acid **1.67** was prepared by a sequence involving a ring closing metathesis.[134]

Scheme 1.13

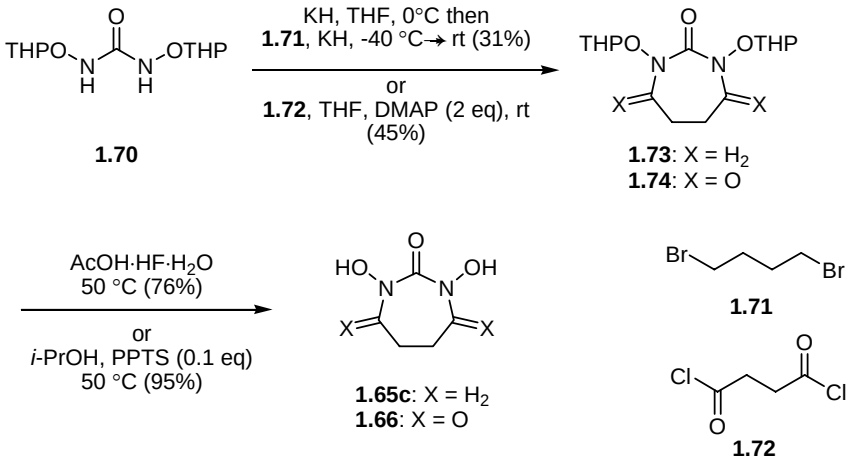


Table 1.2: Results of Kinetic Studies on Cyclic *N, N′*-Dihydroxyureas

Compounds	pK_{a1}, pK_{a1}	K_i (μM) at pH 7.3	K_i (μM) at pH 9.5
1.63	5.6, 7.0	16	23
1.64	5.6, 7.1	no inhibition	no inhibition
1.65a		no inhibition	303
1.65b		no inhibition	146
1.65c	9.4, 11.2	no inhibition	71
1.66		no inhibition	no inhibition
1.67	5.0, 8.7	388	53

As can be seen, a number of *N, N′*-dihydroxyureas were inactive at pH 7.3 but active at pH 9.5. It appeared that at least one of the *N*-hydroxyl groups must be ionized in order for the *N, N′*-dihydroxyurea to serve effectively as a ligand for the zinc ions. Consequently, the pK_as of the two hydroxyl groups play major roles in the potency of *N, N′*-dihydroxyureas as inhibitors.

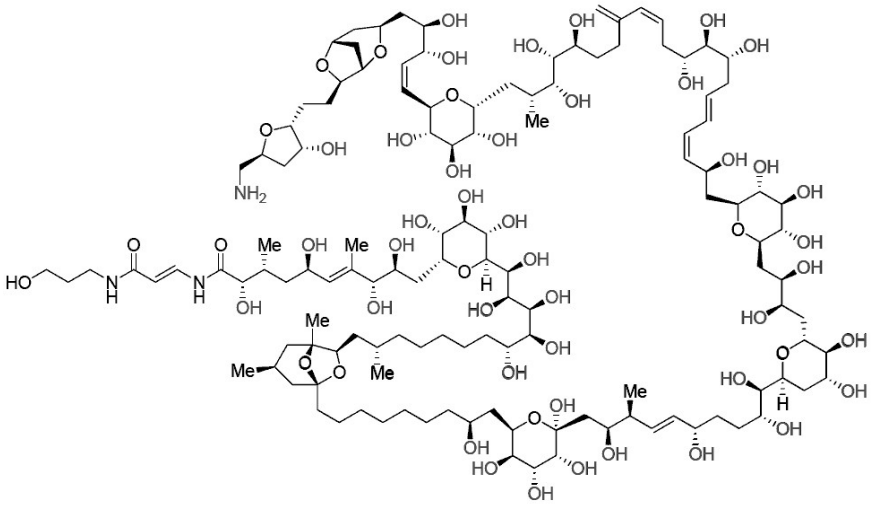
1.4 CONCLUSION

Significant progress has been made in the understanding of the kinetic and mechanistic aspects of the PLC_{Bc}-catalyzed hydrolysis of phospholipids in the past decades. The crystal structure was published; the active site was identified; and a mechanism was proposed based on the kinetic and structural data. On the other hand, little is known about the structure-reactivity relationships in the hydrolytic reaction of phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidyl L-serine (PS), all of which are known natural substrates of PLC_{Bc}. In the upcoming chapter, we will discuss the design of the water-soluble substrate-base analogue, using the X-ray data described in this chapter; we will prepare such inhibitors, using the chemistry discussed here, to gain understanding about the structure-reactivity relationship.

Chapter 2: The Synthesis of *C*-Aryl Glycoside

2.1 *C*-ARYL GLYCOSIDE

The *C*-aryl glycoside antibiotics are a relatively small class of natural products, but they have attracted considerable attention over the past 25 years among synthetic organic chemists and biochemists because of their potent biological activity.[137-140] Furthermore, the incorporation of *C*-glycoside chiral building blocks in the syntheses of biologically important compounds, such as palytoxin (Figure 2.1)[141-143] and halichondrin B,[144] has stimulated the development of new glycosidation methodologies. Modern glycobiology has consistently shown that most biological recognition phenomenon involves the glycosidic part of the cell wall glycoproteins and glycolipids.

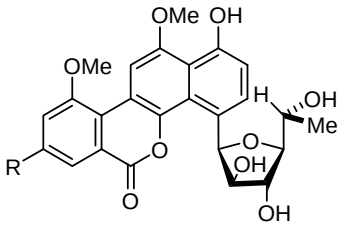


2.1

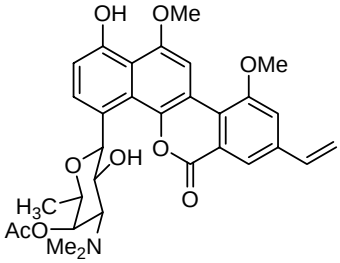
Figure 2.1: Palytoxin Structure

The replacement of the anomeric oxygen of the *O*-glycoside with carbon in *C*-glycoside renders it resistant to both acid hydrolysis and enzymatic hydrolysis by α- and β-glycosidases. Therefore, *C*-glycosides are potential inhibitors of carbohydrate processing enzymes and are stable analogs of glycans involved in important intra- and inter-cellular processes.

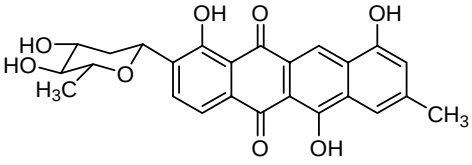
Some representative members of this class of natural products are shown in Figure 2.2. The gilvocarcins (**2.2a-c**)[145, 146] and related compounds ravidomycin (**2.3**) and chrysomycin are metabolites of certain *Streptomyces* species sharing a common tetracyclic aromatic nucleus to which rare sugars are attached as *C*-glycosides at C4. Several of these antibiotics exhibit significant antitumor activity.[147] In particular, gilvocarcin V (**2.2b)** has attracted considerable attention due to its remarkable antitumor activity and exceptionally low toxicity. Its activity can be enhanced with low-energy visible light irradiation.[148] Galtamycinone (**2.4**),[149-151] an unusual member of angucycline family with a linear tetracycle rather than the angular tetracycle structure possessed by most other members, exhibits both antitumor and antibiotic activities. The *Streptomyces*-derived kidamycin antibiotics, including kidamycin (**2.5**), pluramycin and hedamycin, displayed anti-tumor activity.[152] The mechanism of action is believed to involve strong binding to DNA.[153]



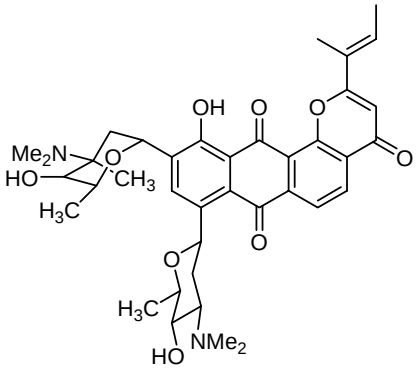
gilvocarcin M (**2.2a**): R = methyl
gilvocarcin V (**2.2b**): R = vinyl
gilvocarcin E (**2.2c**): R = ethyl



ravidimycin (**2.3**)



galtamycinone (**2.4**)



kidamycin (**2.5**)

Figure 2.2: Representing examples of *C*-aryl glycoside antibiotics

Parker categorized *C*-aryl glycosides into four major subgroups based on the orientation of the carbohydrate substituent(s) relative to the phenolic hydroxyl group(s) on the aromatic core.[154] Group I *C*-aryl glycoside has a glycoside linkage *para* to the phenolic hydroxyl; In group II, the linkage is *ortho* to the phenolic hydroxyl. The Group III has two sugars attached to the aromatic ring, one *ortho* and the other *para* to the phenolic hydroxyl. In group IV, the carbohydrate moiety is *ortho* to one of the two dihydroquinone hydroxyls (Figure 2.3).

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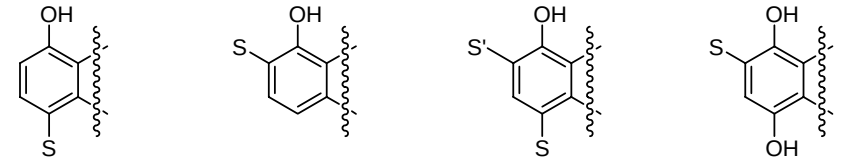


Figure 2.3: Major groups of *C*-aryl glycosides

The two fundamental issues in *C*-aryl glycoside synthesis involve control of the absolute stereochemistry at the anomeric center and control of the point of carbohydrate attachment to the aromatic core. Stereoselective syntheses of *C*-aryl glycosides and *C*-glycosides have been reviewed a number of times in literature, by Postema[140, 155], Knpap[156], Levy[157], Beau[137], Nicotra[138] and Du[139]. Therefore, the discussion in the following sections only serves to provide an overview of the synthetic methods currently available, and the emphasis is upon recently developed approaches.

2.2 THE FORMATION OF C-GLYCOSIDE BONDS

The commonly used routes to *C*-aryl glycosides can be roughly classified into the following four major approaches:

1. Substitution reactions, where the glycosyl donor can be either nucleophilic or electrophilic.
2. Transition metal catalyzed two-partner couplings, especially with palladium catalyst.
3. Pericyclic reactions, most notably hetero-Diels-Alder cycloadditions for the *de novo* synthesis of the carbohydrate moiety of *C*-aryl glycosides.
4. Intramolecular cyclizations, such as ring closing metathesis, cyclotrimerization and electrophilic ring closure.

2.2.1 Substitution With Electrophilic C-Glycosyl Donors

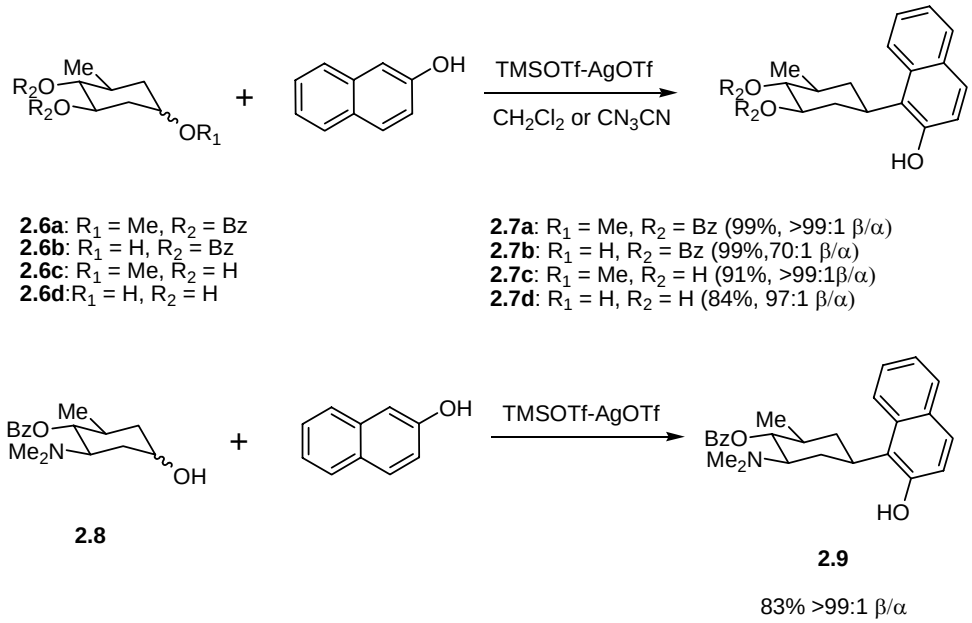
Nucleophilic substitution at the anomeric carbon is by far the most widely used method for glycosidyl bond formation. This approach takes advantage of the inherent electrophilicity of the anomeric carbon. For convenience, reactions involving electrophilic glycosyl donors such as lactol, *O*-glycoside, *S*-glycoside, anomeric ester, halide, imidate, lactones, glycal, 1,2-anhydride and enitol, are included in this category.

2.2.1.1 Anomeric Halides, Esters and Ethers

Glycosyl halides are frequently used as electrophilic donors. Anomeric chlorides, bromides and fluorides have all received considerable attention. Lewis acids are used extensively to enhance the electrophilicity of the anomeric carbon through the formation of oxocarbenium ions, which are trapped with electron-rich arenes to form *C*-aryl glycosides. With pyranose sugars, electrophilic attacks are often from the α-face leading to α-*C*-glycosides. This is due to the anomeric effect of the ring oxygen directing the incoming nucleophile to the α-face. In the more flexible furanose, the steric bias of the two faces usually dictates the product ratio often resulting in forming a mixture. Oxocarbenium intermediates can also be generated from anomeric acetates, trifluoroacetates, *O*-glycosides, *S*-glycosides or lactols. The anomeric leaving group ability dictates the strength of the Lewis acid needed for the reaction

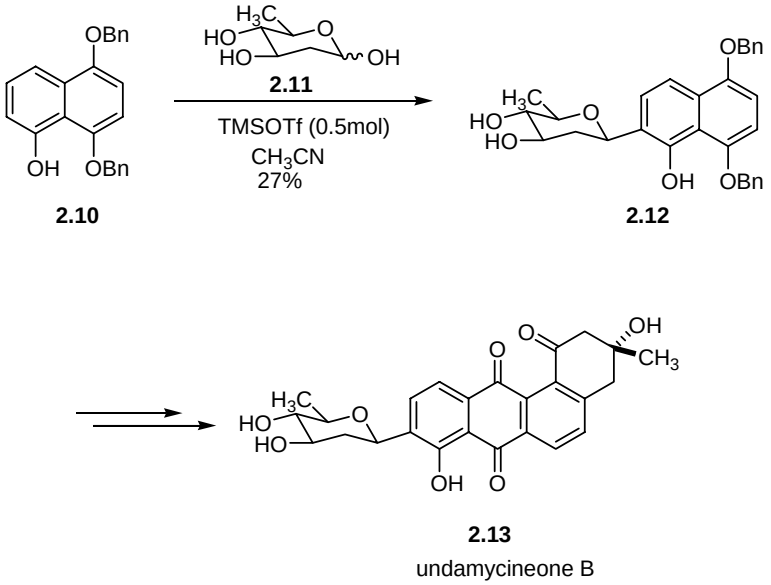
Toshima[158] demonstrated that treatment of pyranose (**2.6b** or **2.6d**) or *O*-methyl pyranoside (**2.6a** or **2.6c**) with TMSOTf/$AgClO_4$ in the presence of 2-naphthol gave excellent yields of the corresponding β-*C*-aryl glycosides **2.7** (Scheme 2.1). The α/β ratios were usually excellent with the lowest being 15:1. Unprotected sugars and amino sugars fared well in the arylation reaction.[159]

Scheme 2.1



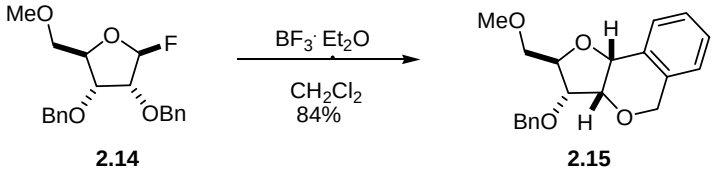
Further optimization revealed TMSOTf alone was an efficient activator in most cases.[160] The reaction process most likely involved an *O*→*C*-glycoside rearrangement as the use of 10% mol promoter gave a considerable amount of *O*-glycosylated product. The high β selectivity resulted from product equilibration, which favors the more thermodynamically stable β anomer. Toshima and coworkers a few years later completed the total synthesis of urdamycinone B (**2.13**) *via C*-glycosidation of naphthol **2.10** with the unprotected D-olivose **2.11** (Scheme 2.2).[161]

Scheme 2.2

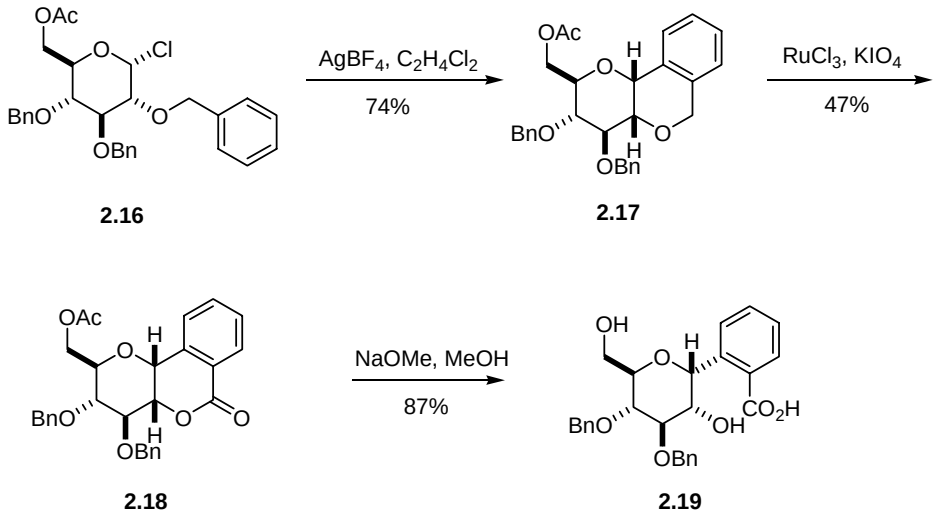


Alternatively, the oxocarbenium could be trapped internally to form a bicycle as demonstrated in Martin's[162-164] and Araki's[165] work (Scheme 2.3). Veyrieres and coworkers[166] prepared D-glucopyranosylbenzoic acid **2.19,** which cannot be accessed through intermolecular reaction due to the presence of an electron-withdrawing carboxylate, from glycosyl chloride **2.16** *via* an intramolecular Friedel-Crafts reaction (Scheme 2.4).

Scheme 2.3



Scheme 2.4

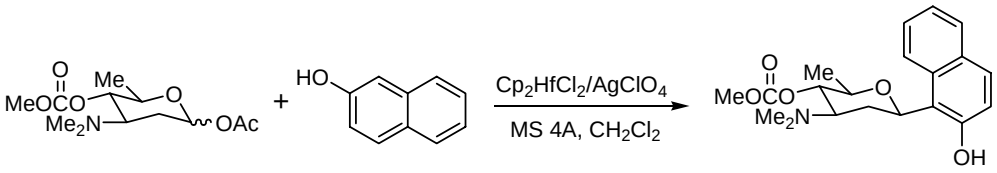


2.2.1.2 Suzuki's Approach to C-Aryl Glycosides

Some of the more elegant aspects of the *O*→*C*-glycoside rearrangement were developed by Suzuki and demonstrated in his syntheses of a number of *C*-aryl glycoside natural products. His group[167] discovered that $Cp_2HfCl_2/AgClO_4$ was a highly effective catalyst for the glycosidation of phenols with glycosyl fluorides at low temperature.

Later development of the *O*→*C*-glycoside rearrangement allowed the use of the more readily available and shelf stable anomeric acetates as glycosyl donors.[168] Suzuki also found that the use of $Cp_2HfCl_2/AgClO_4$ in 1:2 rather than 1:1 ratio provided much higher reactivity for the activation of the glycosyl fluoride. The activating catalyst proposed by the author was $Cp_2Hf(ClO_4)_2$ or $Cp_2Hf(ClO_4)Cl$, depending upon the ratio of Cp_2HfCl_2 and $AgClO_4$.[169] A variety of 2-deoxy-*C*-aryl glycosides including amino sugar derivatives, such as **2.22**, were prepared similarily (Scheme 2.5).

Scheme 2.5

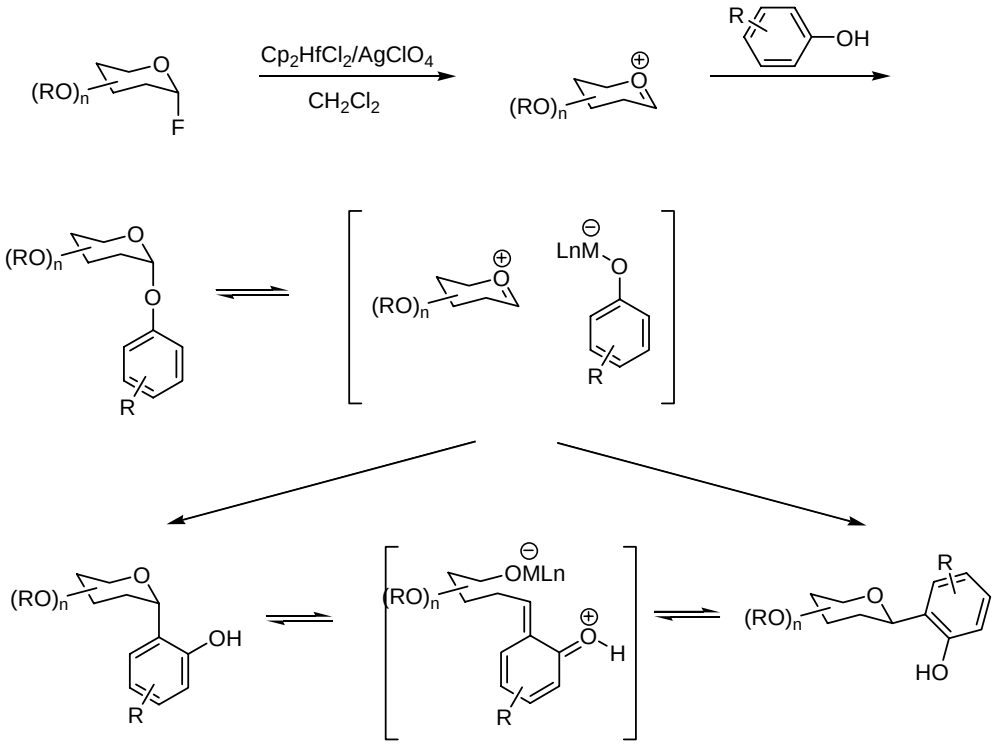


2.20 **2.21** **2.22**

94%, β only

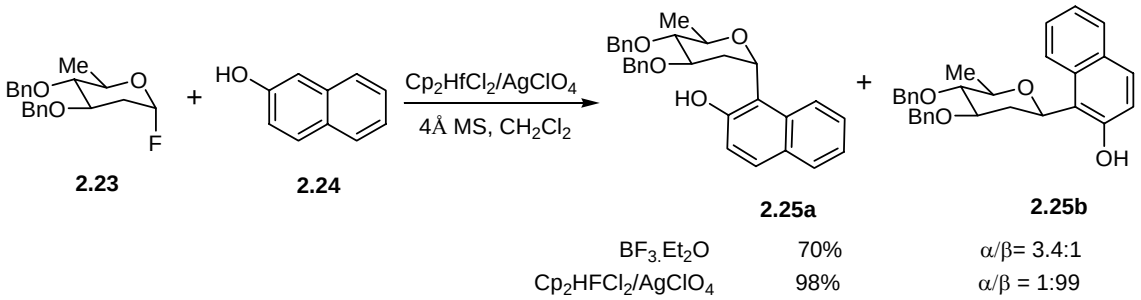
The mechanism proposed by Suzuki involves activation of a glycosyl fluoride by the fluorophilic hafnium complex to generate the reactive oxocarbenium intermediate, which is rapidly trapped by the phenolic hydroxyl group to provide the *O*-aryl glycoside. Upon warming up, the *O*-glycoside rearranges *in situ* in the presence of a suitable Lewis acid into *C*-glycoside. Mechanistic studies suggested that the *O*→*C*-glycoside rearrangement occurred *via* a loose ion pair involving an oxocarbenium,[167] which underwent an irreversible Friedel-Crafts alkylation regioselectively at the carbon *ortho* to the phenolic hydroxyl. This "*ortho* selectivity" of the aryl *C*-glycoside bond formation was generally high, if not excusive.[167] Another important point was the anomeric stereocontrol of *C*-aryl glycoside. The α/β selectivity was determined not only kinetically but also by a possible contribution of an *ortho*-quinone methide intermediate, which resulted in epimerization at the anomeric carbon (Scheme 2.6). The ring opening/reclosure process, if it occurred, would prefer the thermodynamically more stable β-anomer since the anomeric effect was no longer decisive at the stage of the final product.[170] Suzuki concluded that the α-anomer was typically the kinetic product and less sterically congested β-anomer was the thermodynamic product in the *O*→*C*-glycoside rearrangement of pyranoside.[167] It is therefore possible to obtain either anomer as the major product by careful selection of the Lewis acid and reaction conditions.

Scheme 2.6



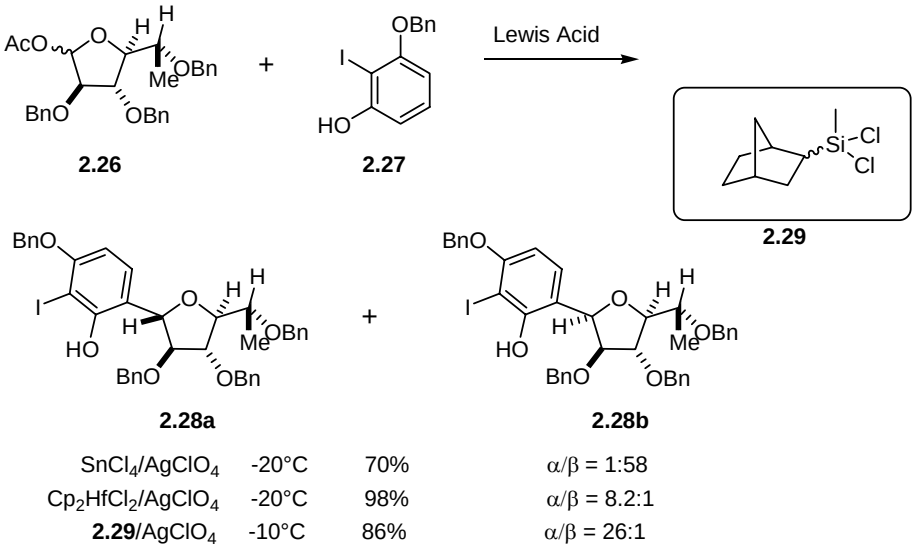
In the synthesis of vineomycinone B_2, Suzuki observed that the $BF_3{\cdot}Et_2O$ mediated *O*→*C*-glycoside rearrangement gave *C*-naphthyl glycoside **2.25** as mixture (3.4:1) of anomers in 70% yield favoring the α anomer. The remaining mass was unrearranged *O*-glycoside, whereas the hafnocene promoter led to almost exclusive β-selectivity in 98% yield (Scheme 2.7).[170]

Scheme 2.7



An impressive reversal of selectivity was observed in the key step of Suzuki's total synthesis of gilvocarcin M (**2.2a**).[146] Glycosyl acetate **2.26** was treated with $SnCl_4/AgClO_4$ in the presence of the resorcinol derivative **2.27** affording high β-selectivity of the *C*-aryl furanoside **2.28** even at low temperature. In sharp contrast, the hafnocene combination $Cp_2HfCl_2/AgClO_4$ gave the desired α-anomer **2.28a** in 86% and 8:1 α/β selectivity. No anomerization was observed upon warming to room temperature. This represented a striking difference since prior studies had established that hafnocene combination $Cp_2HfCl_2/AgClO_4$ often resulted in thermodynamic control. Later optimization found that the combination of a silyl chloride and $AgClO_4$ afforded higher α stereoselectivity, and norbonane derived chlorosilane **2.29** gave the highest selectivity. None of the product distributions, however, corresponded to the 1:1 ratio the author obtained when he equilibrated α- and β-anomer **2.28a** and **2.28b** separately in the presence of protic acid under forcing conditions (Scheme 2.8). The author rationalized the difference based on the relative stability of α- and β-anomer coordinated to the Lewis acid rather than in their free forms.[146]

Scheme 2.8



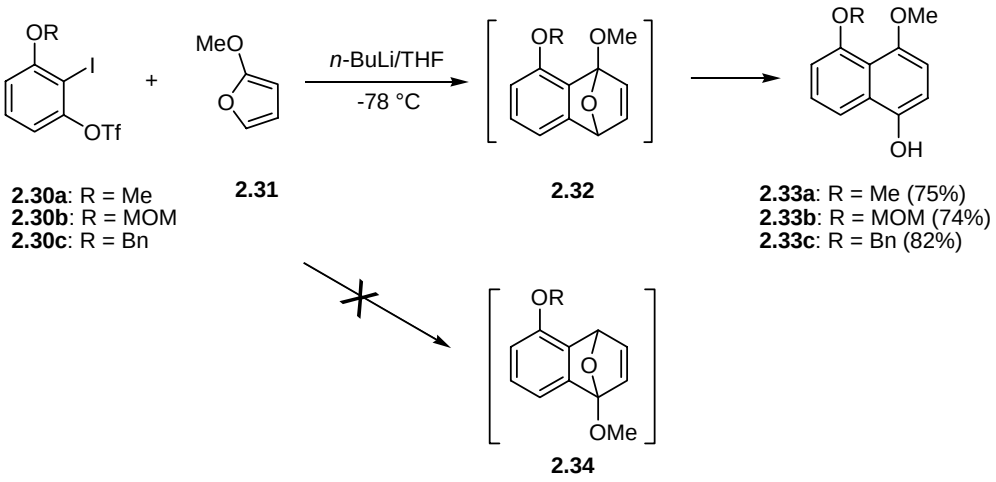
2.26 + **2.27** → **2.29**

2.28a + **2.28b**

$SnCl_4/AgClO_4$	-20°C	70%	α/β = 1:58
$Cp_2HfCl_2/AgClO_4$	-20°C	98%	α/β = 8.2:1
2.29/$AgClO_4$	-10°C	86%	α/β = 26:1

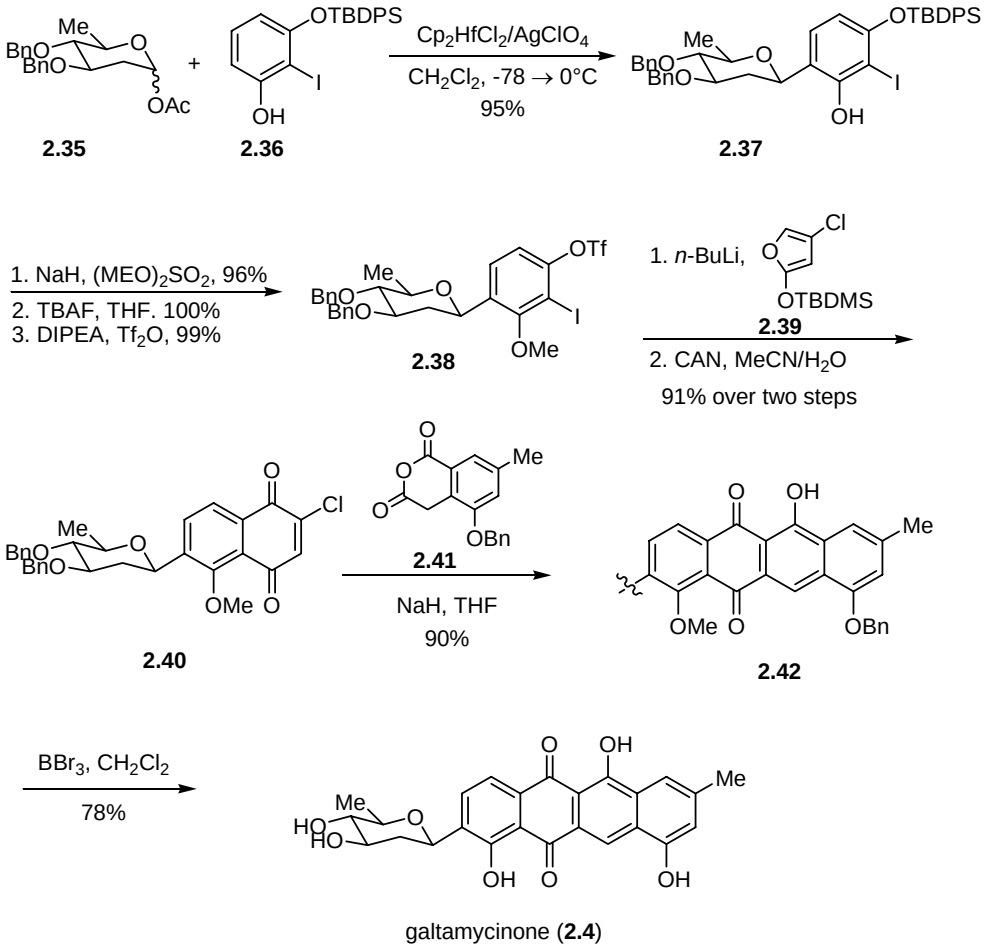
Suzuki also developed a convergent approach to *C*-aryl glycosides that relied on regioselective cycloadditions of glycosyl-substituted α-alkoxybenzynes and alkoxy- or silyloxyfurans.[171, 172] The requisite glycosyl benzynes were obtained through functionalization of *C*-aryl glycosides from the *O*→*C*-glycoside rearrangement. The [2+4]-cycloadditions of α-alkoxyarynes and 2-methoxy- or 2-siloxy-furans proceeded regioselectively in the head-to-head manner. No head-to-tail adducts were detected (Scheme 2.9). This regiochemical mode of cycloaddition can be attributed to the polar effect of the alkoxy substituent in the aryne intermediate.

Scheme 2.9



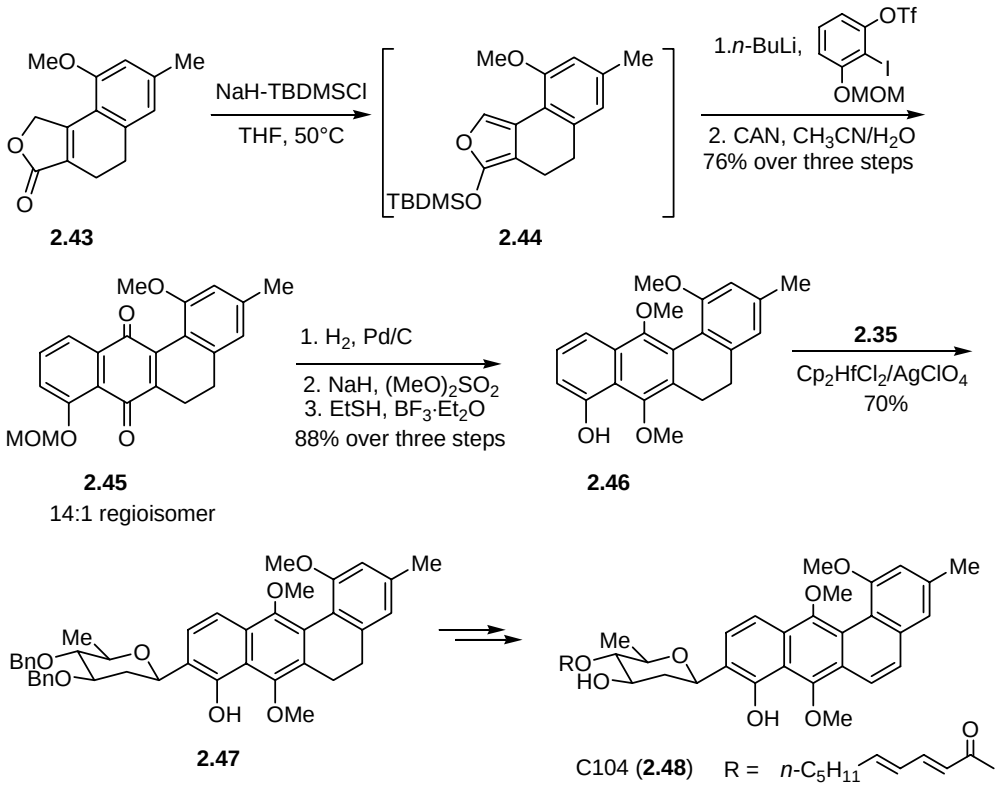
A number of natural products have been prepared using *O*→*C*-glycoside rearrangement and α-alkoxybenzyne/alkoxyfuran cycloaddition.[145, 151, 173-177] In his synthesis of Group II *C*-aryl glycoside antibiotic galtamycinone (**2.4**),[151, 178], Suzuki treated anomeric acetate **2.35** with $Cp_2HfCl_2/AgClO_4$ in the presence resorcinol **2.36** to obtain *C*-glycoside **2.37**. The two hydroxyl functional groups on the aromatic ring were then elaborated to afford the benzyne precursor **2.38**. Regioselective cycloaddition of alkoxybenzyne from **2.38** and siloxyfuran **2.39** followed by oxidation afforded *C*-glycosyl juglone **2.40** in high yield. The chloroquinone **2.40** was then cyclized with the enolate of anhydride **2.41** in highly regioselective manner to give, after spontaneous decarboxylation, naphthacenequinone **2.42** in 90% yield. The protecting groups were removed globally on exposure to BBr_3 at –78 ° C to afford galtamycinone (**2.4**) (Scheme 2.10).

Scheme 2.10



The total synthesis of antibiotic C104 (**2.48**) represents a more daring application of these tactics.[175, 179] Siloxyfuran **2.44**, prepared *in situ* from butenolide **2.43**, was cyclized with the benzyne generated from **2.30b**. The cycloadduct was oxidatively worked up to afford quinone **2.45** (head-to-head cyclization) and its head-to-tail regioisomer in 14:1 ratio. Reduction and bismethylation of quinone **2.45** followed by removal of the MOM protecting group afforded phenol **2.46**. Phenol **2.46** was converted to β-C-glycoside **2.47** upon reaction with D-olivosyl acetate **2.35** in the presence of the hafnocene promoter**,** which was then converted to antibiotic C104 (**2.48**) (Scheme 2.11).

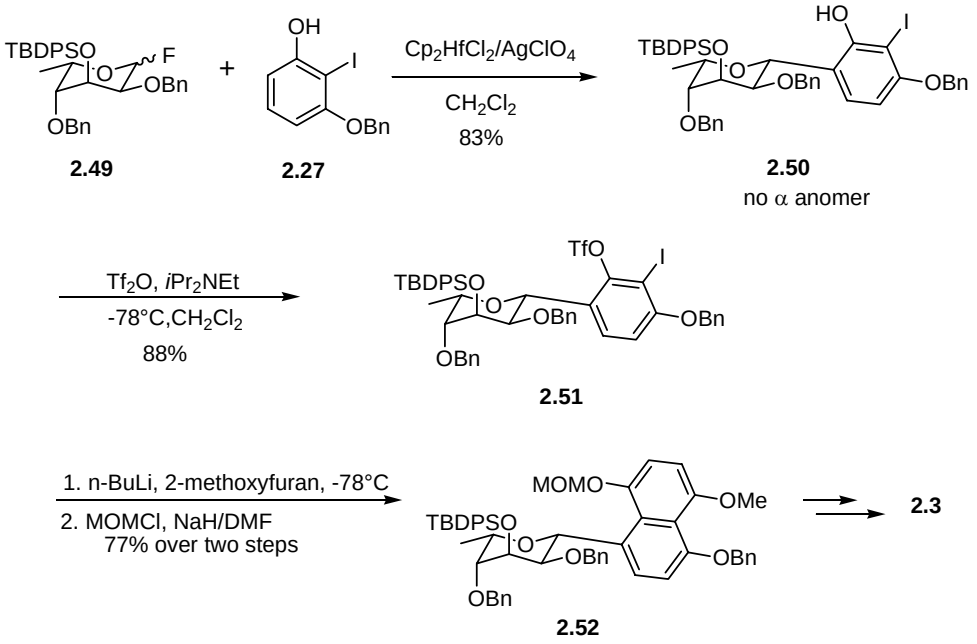
Scheme 2.11



Suzuki also applied the strategy to the syntheses of Group I *C*-aryl glycoside antibiotics, such as ravidomycin (**2.3**).[173, 180] The presence of *C*-glycosidic bond at the position *para* to a phenolic hydroxyl ravidomycin was a serious obstacle to the adoption of the tactics previously described. Hence, Suzuki translated this "*ortho* selectivity" in *O*→*C*-glycoside rearrangement into the "*para* selectivity" by applying the reaction to monoprotected resorcinol **2.27**. Hafnocene promoted glycosidation of **2.27** with glycosyl fluoride **2.49**, whose large *tert*-butyldiphenylsilyl protective group favored the axial disposition to avoid unfavorable gauche interaction when disposed equatorially, gave β-*C*-glycoside **2.50** with the additional oxygen functionality at the *para* position.[181] Phenol **2.50** was then converted to its triflate **2.51** with trifluoromethanesulfonic anhydride.

Treatment of **2.51** with *n*-BuLi generated the reactive benzyne, which was trapped by 2-methoxyfuran to provide the corresponding cycloadduct. Spontaneously opening of the cycloadduct during aqueous work-up and subsequent protection of the naphthol hydroxyl as its MOM ether afforded *C*-naphthyl glycoside **2.52** in 77% yield over 3 steps (Scheme 2.12), which was then converted into ravidomycin successfully (**2.3**).

Scheme 2.12

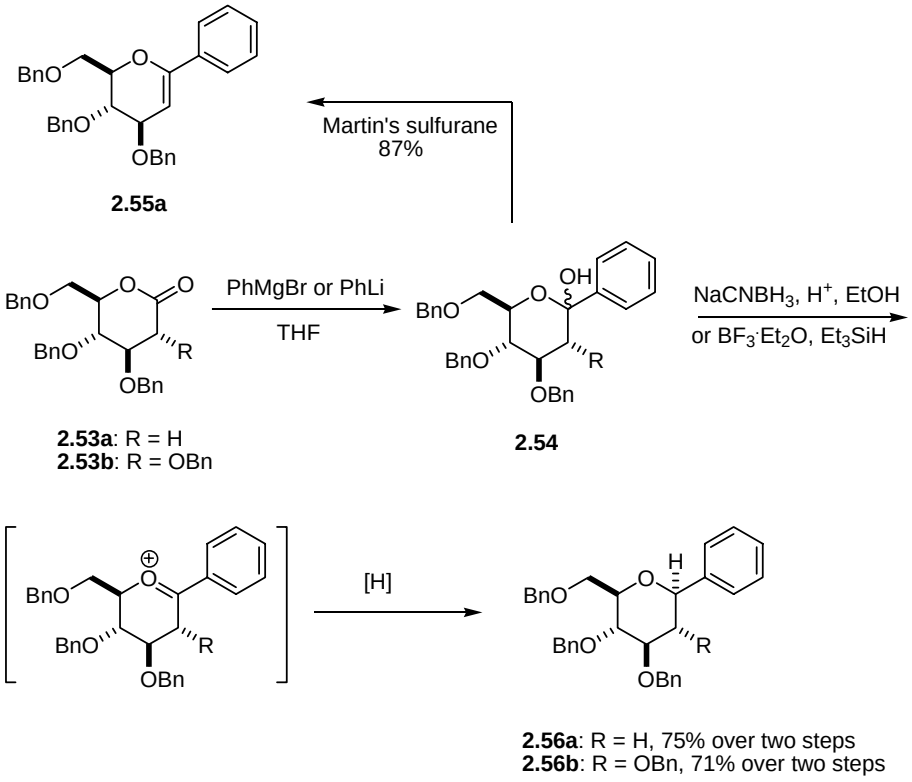


2.2.1.3 C-1 Lactones

Carbohydrate-derived lactones are excellent electrophiles. Organometallic reagents, in particular organolithium and Grignard reagents, are used frequently for the synthesis of hemiacetals/lactols of the general type **2.54** *via* nucleophilic addition to lactones **2.53**. Stereoselective hydride reduction of **2.54** under Brønsted[182] or Lewis acidic conditions[183, 184] furnishes β-*C*-aryl glycosides **2.56**, typically in good yields and excellent stereoselectivities. This represents one of the more reliable methods for

synthesizing gluco- and galacto-β-*C*-aryl glycosides. Ellsworth recently examined a number of commercially available organosilanes for their abilities as reducing reagents, and he found that the critical factor promoting β selectivity was the steric bulk surrounding the silyl hydride center.[185] The highest β selectivity was obtained with triisopropylsilane. Alternatively, dehydration of the hemiacetal intermediate derived from 2-deoxy sugar, such as **2.53a**, with Martin's sulfurane ($Ph_2S[OC(CF_3)_2Ph]_2$) affords *C*-aryl glycal (Scheme 2.13).[182]

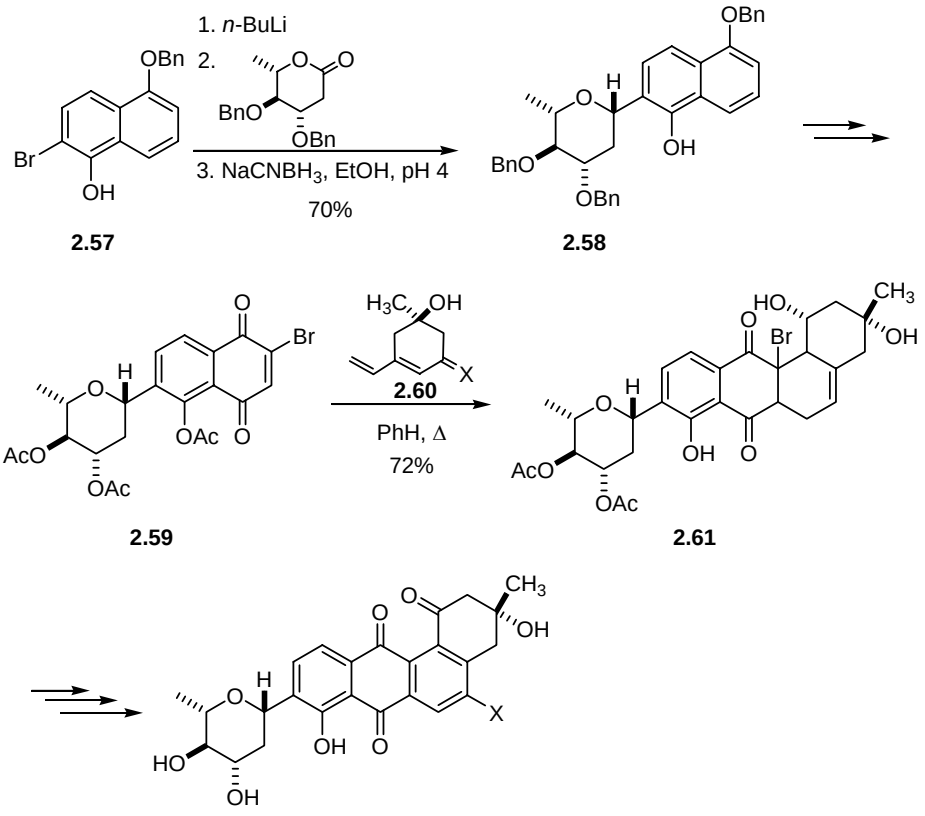
Scheme 2.13



Sulikowski obtained the key intermediate **2.58** *via* addition of aryllithium to a sugar lactone followed by reduction of resultant lactol with acidic sodium cyanoborohydride in his total syntheses of the angucycline antibiotics urdamycinone B

(**2.62a**) and 104-2 (**2.62b**).[182, 186] Another key step was the Diels-Alder cycloaddition between diene **2.60** and the bromojuglone **2.59** to assemble the C and D rings (Scheme 2.14).

Scheme 2.14



2.2.1.4 1,2-Anhydrosugars

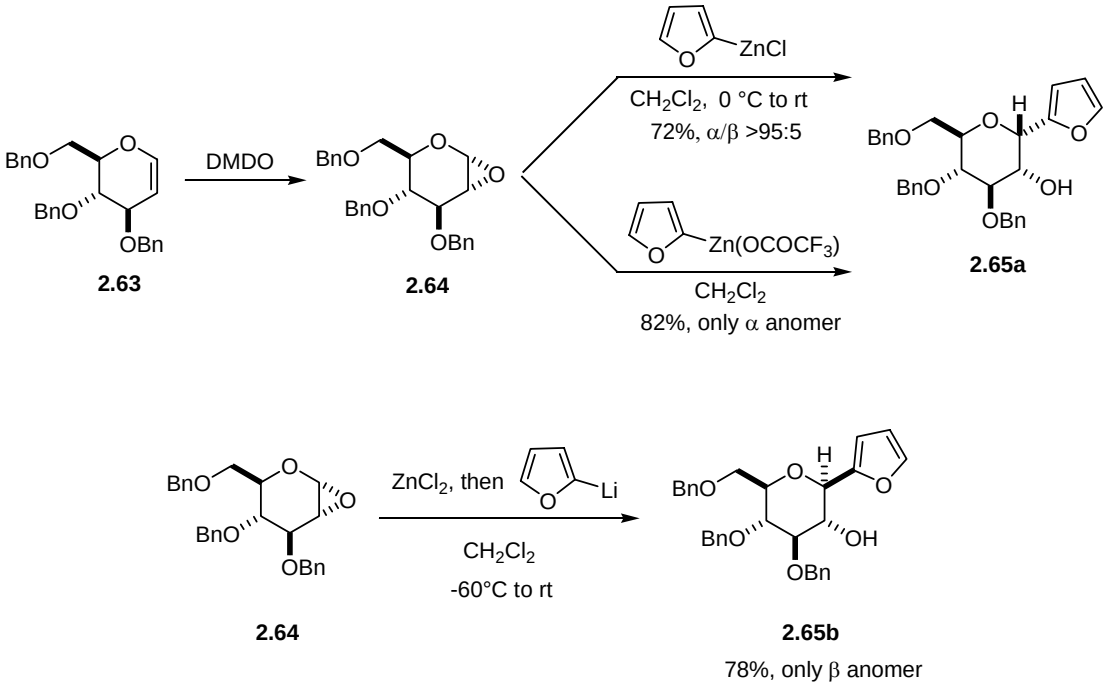
1,2-Anhydrosugars have also served as electrophilic glycosyl donors. These epoxides[187] are mostly generated *via* stereoselective epoxidation of the corresponding glycals with dimethyldioxiran (DMDO) under very mild conditions.[188] No purification is required except for evaporation of the solvents prior to the addition of carbon

nucleophiles. Various organometallic reagents have been used to open the glycal epoxide. Among them, Grignard reagents,[189-191] organocuprate,[192, 193] and organostannanes[194] generally prefer to give β-*C*-glycosides, where the C2 hydroxyl and the newly formed anomeric carbon-carbon bond are *trans* to each other.

Only until recently were methods preferentially providing the α-glycoside starting to emerge. Rainier utilized triarylaluminum- and triarylboron-reagents to open 1,2-anhydrosugars to yield 1,2-*cis*-α-*C*-aryl glycoside with high stereoselectivity.[195] The overall stereochemistry at the anomeric center was retained after the reaction. The author proposed the *syn* opening was due to complexation of aluminum or boron to the oxirane oxygen followed by "intramolecular" ligand delivery to an oxocarbenium intermediate. Thus, it is now possible to obtain either α- or β-*C*-glycoside from a single glycosidic donor by choosing the proper organometal reagent.

More recently, Xue observed high α-selectivity in the reaction of 1, 2-anhydrosugar with an organozinc reagent.[190] Treatment of tri-*O*-benzyl-D-glucal epoxide **2.64** with organozinc trifluoroacetate generated from the corresponding dialkyl- or diarylzinc and trifluoroacetic acid, or preformed organozinc chloride afforded the α-*C*-aryl glycoside **2.65a** with almost complete stereocontrol. However, Rainier reported earlier that when zinc chloride and 1-lithiofuran, rather than preformed furylzinc chloride, was used in the reaction at colder temperature (-60°C), β-glycoside **2.65b** was the only product formed (Scheme 2.15).[191] Xue made no attempt to rationalize the reversed stereoselectivity.

Scheme 2.15

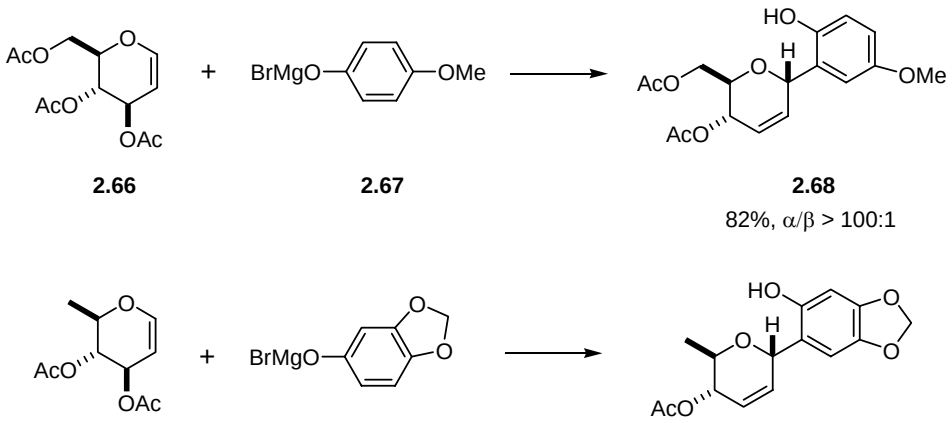


2.2.1.5 Glycal and the Ferrier Rearrangement

The vinylougous version of the electrophilic substitution, where the leaving group is located at allylic C3 position rather than the anomeric carbon, is typically referred to as the Ferrier rearrangement.[196] Though the original version of Ferrier rearrangement limited itself to oxygen nucleophiles and hence the synthesis of *O*-glycoside. Carbon nucleophiles, such as electron rich aromatics, can nonetheless be used. The reaction usually proceeds through a vinyl oxocarbenium intermediate, and subsequent Friedel-Crafts arylation affords *C*-aryl glycoside. There have not been as many successful examples in this area of research. The α/β stereoselectivity is not often prominent due to the more flexible intermediate and nucleophilic attack at the C3 allylic position poses an additional obstacle especially when softer carbon nucleophiles are used.[197]

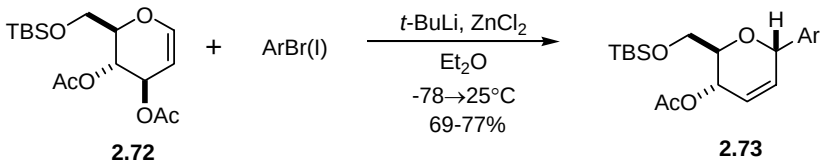
Casiraghi developed a highly regio- and diastereoselective approach to 1-*C*-aryl-2,3-ene-*a*-D-glycosides,[198, 199] such as **2.68** and **2.71**, *via* direct arylation of acetylated pyranoid glycals at *C*-l using bromomagnesium phenolates in dichloromethane (Scheme 2.16). It was necessary, however, to use large excess (typically four equivalents) of the bromomagnesium phenolate combined with ultrasonic irradiation to achieve successful arylation in the heterogeneous mixture. The reaction was regiospecific with respect to both the arylation and the substitution site, with the glucal anomeric carbon being arylated and the phenol *ortho*-carbon being substituted. In addition, the reaction was stereoselective with α-anomer being produced predominantly or exclusively, as a result of kinetic discrimination of the two diastereotopic faces of the transition state, wherein the aromatic *ortho*-carbon preferentially entered the anomeric carbon from the axial direction at *C*-l and *anti* to the 3- and 5-substituents according to a S_N2' anti-selective process.[199] The reaction was sensitive to the disposition of the C4-acetoxy group adjacent to the leaving group, therefore the method was not applicable to the syntheses of sugars having a 3,4-*cis* relationship.

Scheme 2.16



Du Bois[200] and coworkers recently disclosed a procedure involving nucleophilic addition of organozinc to 1,2-dihydropyranyl-3-acetate to produce Ferrier type product in good yield with high α selectivity (Scheme 2.17). The broad compatibility of the organozinc nucleophiles represented an advantage.

Scheme 2.17

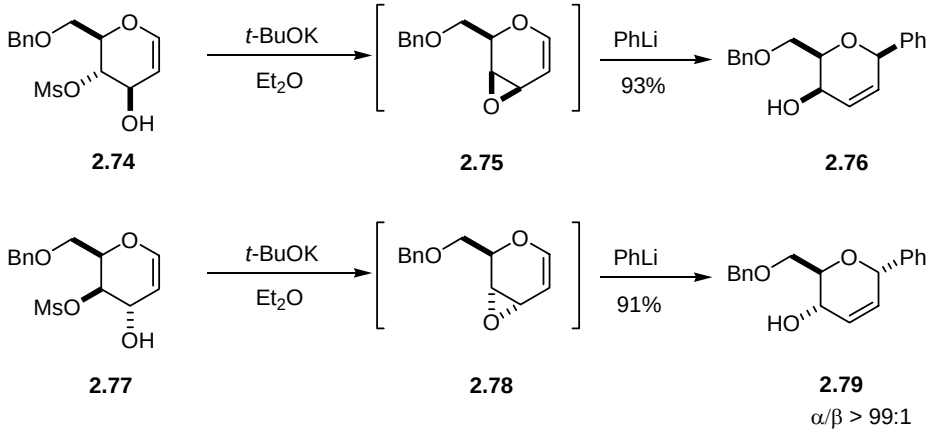


More recently, Minehan reported the uncatalyzed addition of triarylindium to glycal acetates.[201] Couplings were performed between a variety of arylindiums possessing both electron-donating and -withdrawing substituents and glucal-, rhamnal- and galactal-acetates and the yields were good in most cases. However, the stereoselectivity (α/β ratio) was only moderate (5:1) for glucal- and rhamnal-acetate.

Crootti reported that treatment of 1,2-unsaturated-3,4-anhydroglucals (pseudoglucal) **2.75** and **2.78**, generated *in situ* from D-glucal derived hydroxy mesylates **2.74** and **2.77**, with aryllithium reagents afforded 2,3-unsaturated-*C*-glycosides **2.76** and **2.79**, respectively, with excellent 1,4-regioselectivity (Scheme 2.18**)**.[202, 203] The 1,4-displacement was highly stereospecific with α- and β-epoxide leading to the exclusive formation of α- and β-*C*-glycoside, respectively. It was necessary to generate the reactive vinyl oxiranes *in situ* in a separate step since direct treatment with PhLi only yielded a complex mixture. Other organometals, such as Grignard reagent, organocuprate and organozinc, displayed more complex behavior in their reactions with epoxides **2.75** and **2.78**. The author explained the complete yet opposite stereoselectivity of α and β-

oxiranes based on a model where chelation of the lithium ion with the oxirane oxygen prompted delivery of aryl nucleophile from the same face as oxirane oxygen, resulting in 1,4-*cis* configuration in the final product.

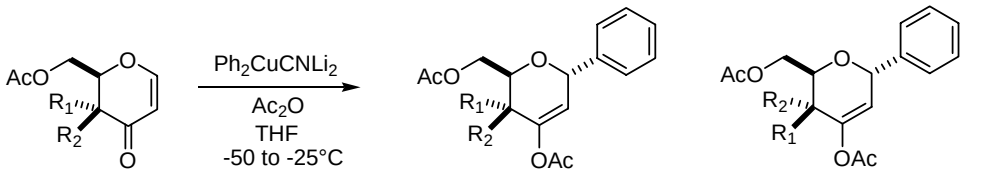
Scheme 2.18



2.2.1.6 Enitols and Michael Type Conjugate Addition

Enones, also known as 1,5-anhydroenitols, have been used successfully in the synthesis of *C*-aryl glycosides, with or without a transition metal catalyst. Bellosta and Czernecki[204] illustrated that Michael type addition of a higher order cyanoarylcuprate to 1,5-anhydro-1-enitol **2.80b** in the presence of acetic anhydride afforded the corresponding α-*C*-aryl glycoside **2.81b** in 87% yield with complete stereocontrol. The author also observed a small amount of C4-epimerization when **2.80a** was subjected to the same chemical transformation (Scheme 2.19). The high facial selectivity was rationalized based on a chair-like transition state where the nucleophile approached from the α face for an axial attack.

Scheme 2.19



2.80a: R_1 = H, R_2 = OAc
2.80b: R_1 = OAc, R_2 = H

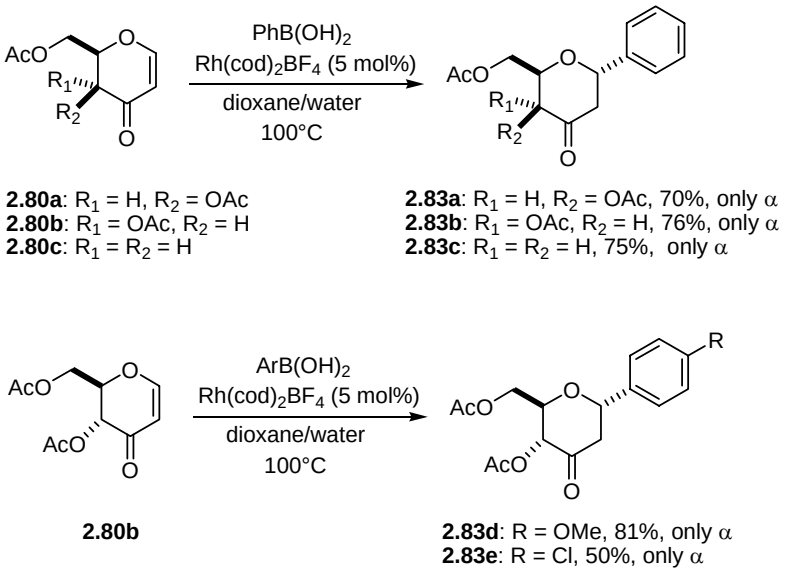
2.81a: R_1 = H, R_2 = OAc, 83%
2.81b: R_1 = OAc, R_2 = H, 87%, only α

2.82a: 3%
2.82b: not observed

A few years later, Czernecki reinvestigated the same reaction[205] using $Pd(OAc)_2$ as catalyst and benzene as glycosyl acceptor. The author observed complete lack of C4-epimerization and exclusive formation of α-*C*-aryl glycoside. However, the reaction was complicated by simultaneous formation of arylated enone attributed to isomerization of intermediate organopalladium species and subsequent *syn* β-hydride elimination.

More recently, Maddaford[206] synthesized *C*-aryl glycoside using the Rh(I)-catalyzed 1,4-addition of an arylboronic acid to 1,5-anhydroenitol. Heating a dioxane/water solution of enones **2.80a-c** and phenylboronic acid in the presence of cationic rhodium catalyst $Rh(cod)_2BF_4$ delivered α-*C*-phenylglycosides **2.83a-c** exclusively in good yields. The reactions proceeded smoothly with boronic acids bearing either electron-donating or electron-withdrawing substituents. No epimerization at C4 was observed and acid/base labile protecting group could be used (Scheme 2.20). The mechanism involved initial transmetalation of the aryl group from boron to rhodium and the organometallic species then added stereoselectively to the α-face of the enone double bond with subsequent hydrolysis of the Rh-O bond. The reaction products could be readily converted into 3-deoxy sugars by stereoselective reduction or to 3-aminosugars by reductive amination.

Scheme 2.20



2.80a: R_1 = H, R_2 = OAc
2.80b: R_1 = OAc, R_2 = H
2.80c: R_1 = R_2 = H

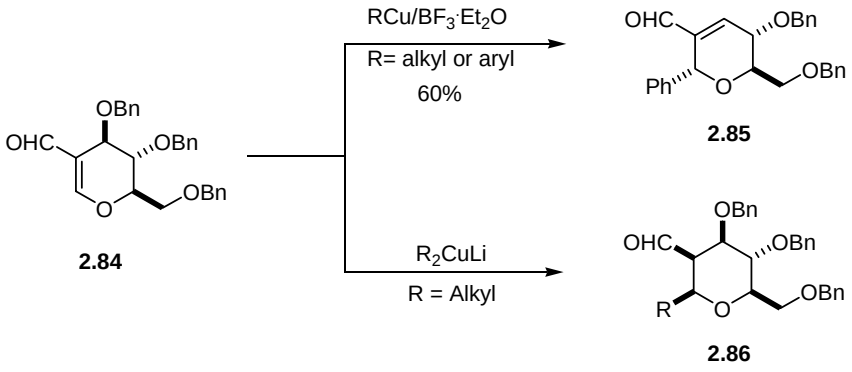
2.83a: R_1 = H, R_2 = OAc, 70%, only α
2.83b: R_1 = OAc, R_2 = H, 76%, only α
2.83c: R_1 = R_2 = H, 75%, only α

2.80b

2.83d: R = OMe, 81%, only α
2.83e: R = Cl, 50%, only α

Lately, Cossy[207] reported an interesting regio- and stereoselective synthesis of α-*C*-aryl glycoside **2.85** and β-*C*-alkylglycoside **2.86** from the same C2-formyl glycal **2.84** using arylcuprates and lithium dialkylcuprates, respectively (Scheme 2.21).

Scheme 2.21



2.84

2.85

2.86

2.2.2 Substitution With Nucleophilic C-Glycosyl Donors

Some researchers have reversed the intrinsic polar nature of the anomeric carbon from electrophilic to nucleophilic to generate a nucleophilic glycosyl donor and trapped it with appropriate carbon electrophiles to produce *C*-aryl glycosides (the "umpolung" strategy). This can typically be done in two ways: 1) Direct and indirect metallation of the anomeric carbon, including lithiation, reductive metallation or transmetallation. 2) Installation of a strongly electron-withdrawing group, such as nitro, carbonyl functionality, sulphone or sulfoxide, at anomeric carbon to lower the pK_a of the anomeric proton. The latter method has found more applications in the synthesis of *C*-alkyl glycosides, and it is less applicable to the syntheses of *C*-aryl glycosides.

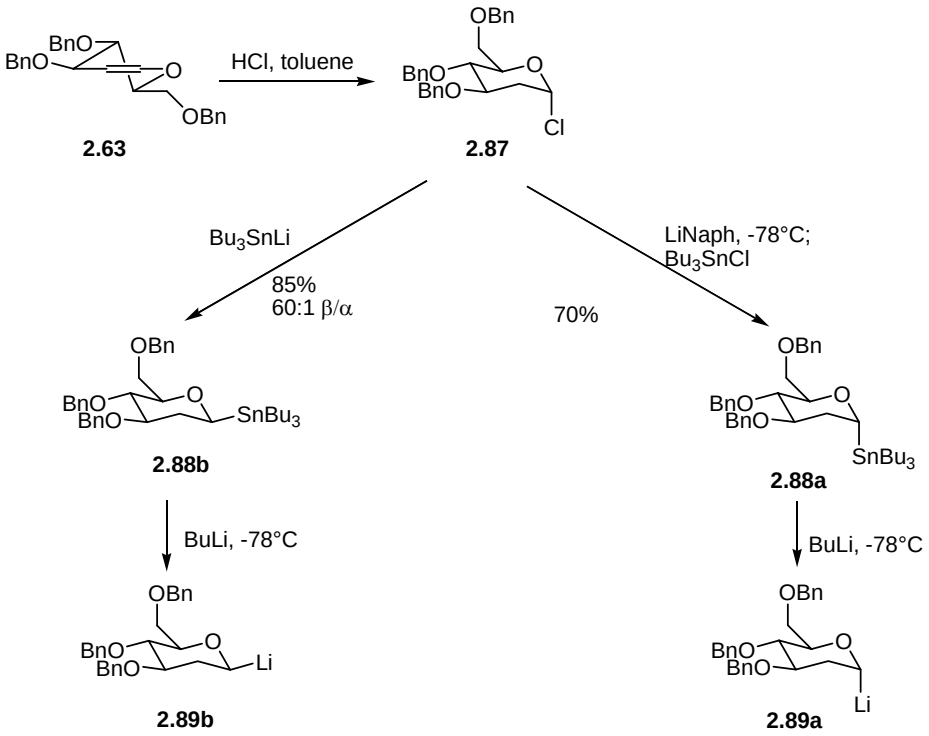
2.2.2.1 C-1 Alkyl Lithium and Alkylstannane

Sinay[208] has developed an approach for reversing the polar nature of the anomeric center to gain access to both the α and β-anomers of *C*-glycoside from a common precursor. Treatment of the unstable 3,4,6-tri-*O*-benzyl-2-deoxy-D-arabino-hexopyrosyl chloride **2.87** with tributylstannyllithium in THF at 0 °C provided the β–glycosylstannane **2.88b** in 85% yield with 60:1 β/α selectivity *via* a likely direct S_N2 displacement. Reductive lithiation of the same glycosyl chloride **2.87** with two equivalents of lithium naphthalenide in THF at -78°C, followed by the quenching with tributyltin chloride provided the α–glycosylstannane **2.88a** in 70% yield. The stereoselectivity results from reduction of the transient axial anomeric radical stabilized by anomeric effect under kinetic control to α-glycosyllithium, an anomeric anionic species that is stereochemically stable at -78°C but has a tendency to equilibrate to the more stable β-anomer upon warming up.[209] These glycosylstannanes bear the advantage of being configurationally stable at room temperature; and they are generally stable to

silica chromatography and can be carried many steps into natural product syntheses (Scheme 2.22).

Cleavage of the tin-carbon bonds in **2.88a** and **2.88b** with BuLi at low temperature occurred rapidly with retention of configuration to give the α-glycosyllithium **2.89a** and β-glycosyllithium **2.89b** respectively. Sinay successfully trapped the anomeric organolithiums using external carbon electrophiles with complete retention of stereochemistry (Scheme 2.22).[210]

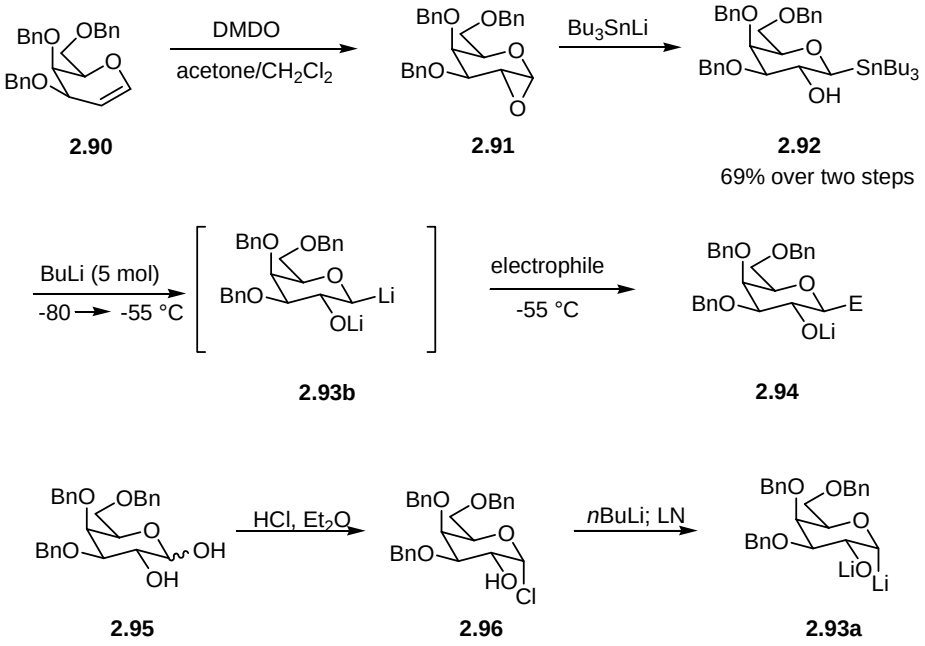
Scheme 2.22



This approach, while providing a rapid access to both α and β-anomers of *C*-glycoside from a common precursor, is limited to 2-deoxy sugars. Elimination to form glycal presents a serious problem when a 2-oxygenated functionality is present. Kessler and coworkers avoided β-elimination by leaving the C2-hydroxy unprotected.[211, 212] The

presence of C2-lithium alkoxide effectively prevents the elimination thus blocking the formation of undesired glycal. Hence, dianions **2.93a** and **2.93b** were generated from vicinal halohydrin **2.96** and β-glycosylstannane **2.92** to provide access to α- or β-*C*-glycoside, respectively. The author was also able to effect similar transformations on 2-amino sugars (Scheme 2.23).[211]

Scheme 2.23

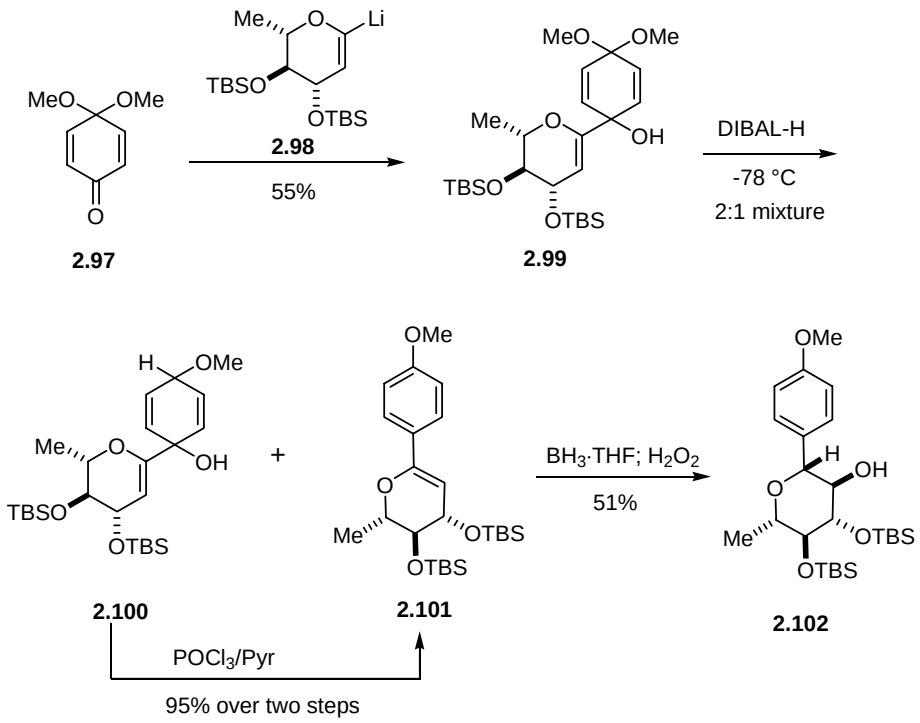


2.2.2.2 Lithioglycal-Parker's Approach to C-Aryl Glycoside

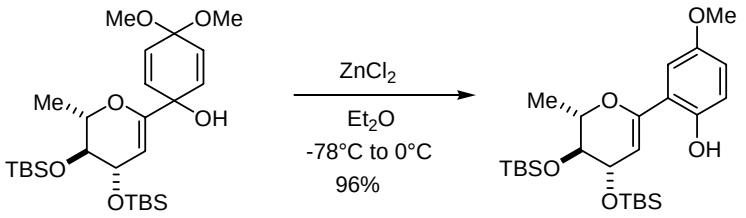
Parker developed a "reversed polarity strategy" for the synthesis of *C*-aryl glycoside synthesis that relied on the addition of a lithio glycal to a quinol ketal or quinone followed by subsequent reductive or non-reductive aromatization.[213] For example, treatment of ketal **2.99**, obtained from the addition of lithiated glycal **2.98** to quinone ketal **2.97**, with DIBAL-H gave a mixture (2:l) of methyl ether **2.100** and aromatized product **2.101**. Exposing the mixture to $POCl_3$ in pyridine afforded **2.101** in

95% yield. Subsequent stereoselective hydroboration/oxidation with borane afforded the desired *C*-aryl glycoside **2.102** (Scheme 2.24). Alternatively, hydrogenation of *C*-aryl glycal **2.101** provided 2-deoxy *C*-aryl glycoside.[214] The position of attachment of the carbohydrate to the aromatic core is determined by the quinol ketal starting material. Benzoquinone could also be used[214] when sodium dithionite was employed in the reductive aromatization step. Alternatively, when the intermediate glycal quinine ketal **2.99** was treated with $ZnCl_2$ in Et_2O, the rearranged *C*-aryl glycoside **2.103** was obtained (Scheme 2.25). Hence, both Group 1 and Group IV *C*-aryl glycosides can be accessed from a common intermediate **2.99**.

Scheme 2.24



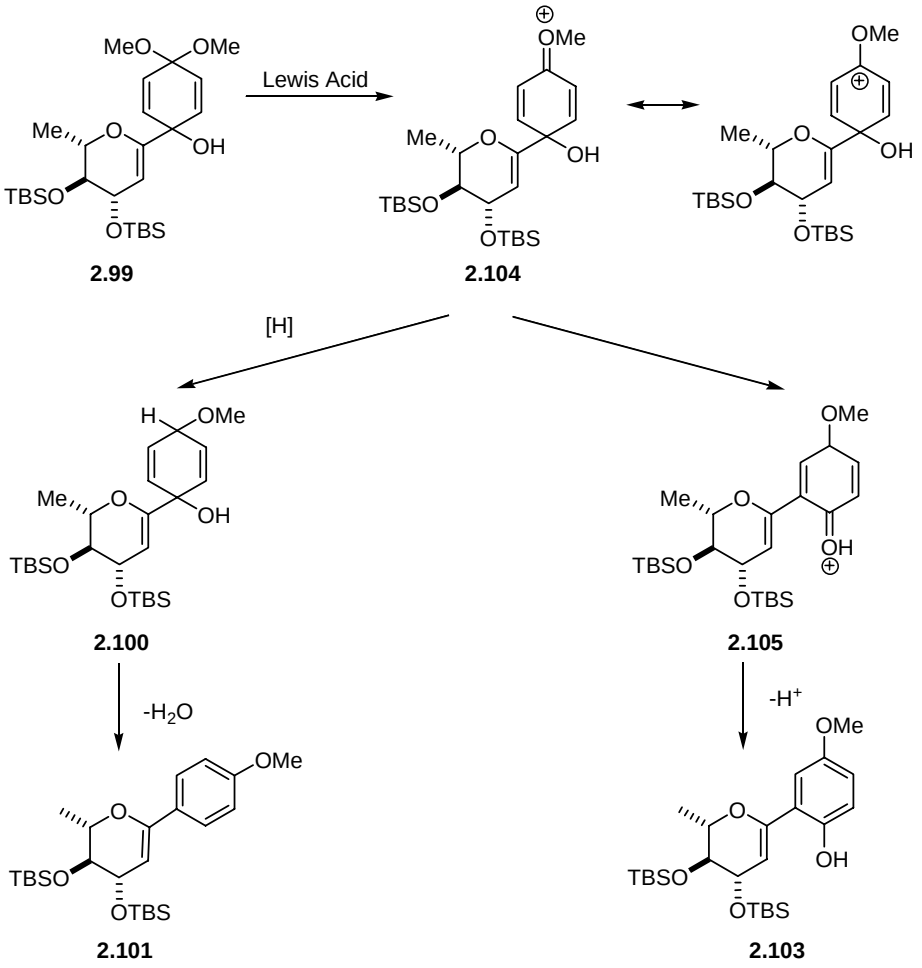
Scheme 2.25



2.99 **2.103**

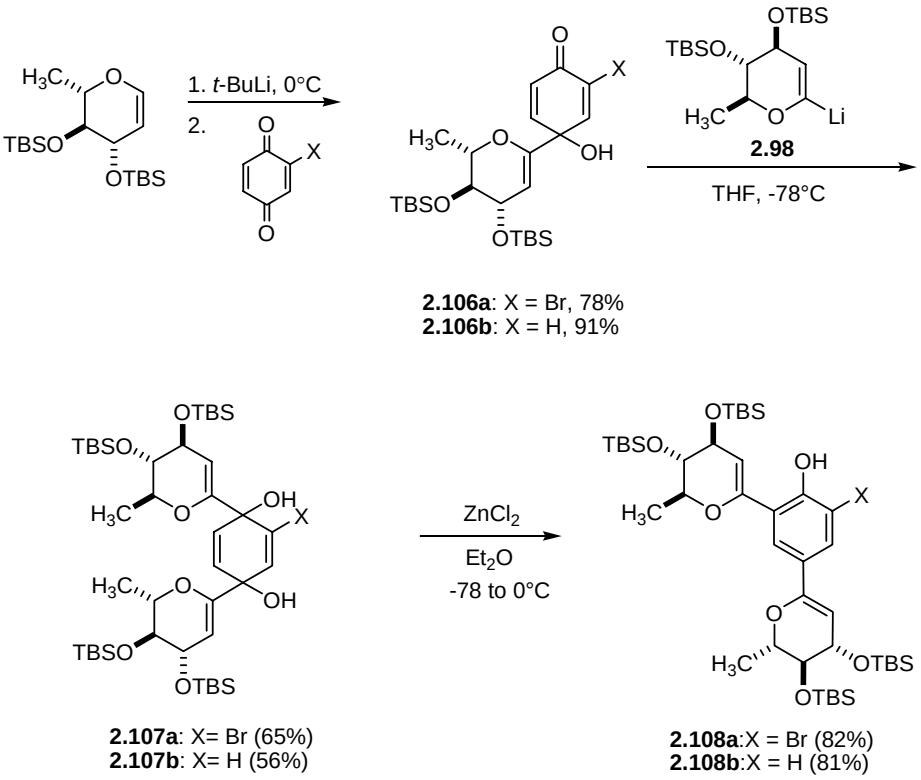
Parker proposed that both reductive aromatization and rearrangement (non-reductive aromatization) arose from a common oxocarbenium intermediate **2.104**. Trapping with a hydride afforded **2.100** and subsequent loss of water afforded aromatic **2.101**. Alternatively, dienone-phenol rearrangement followed by loss of a proton would give **2.103** (Scheme 2.26).[215]

Scheme 2.26



Parker also developed an approach to Group III bis-*C*-aryl glycoside by sequential addition of lithiated glycals to quinone followed by non-reductive aromatization (Scheme 2.27). Hence addition of lithiated glycal **2.98** to either quinol **2.106b** or its bromo counterpart **2.106a**[216] afforded 1,4-diols **2.107b** or **2.107a** in moderate yields. 1,2-Glycal shift took place upon treatment with $ZnCl_2$ providing the two kidamycin model compounds, **2.108b** and **2.108a** in excellent yields. Complete regiocontrol in the rearrangement of bromo substrate **2.107a** to bisglycal **2.108a** was observed.[213]

Scheme 2.27



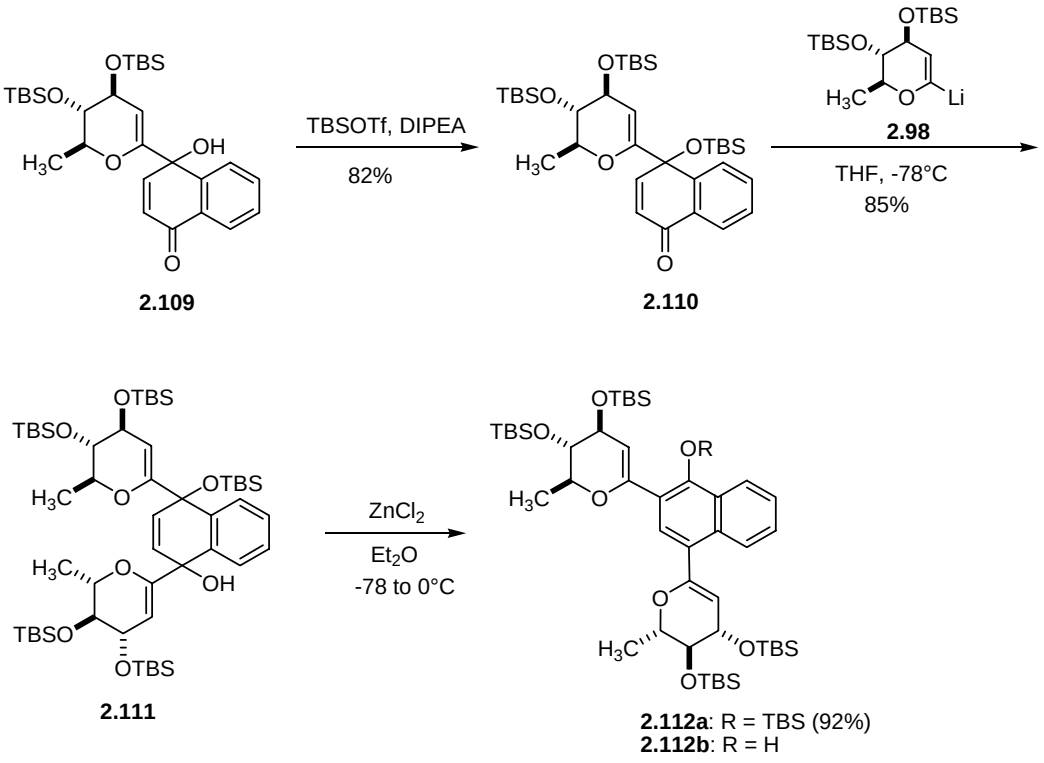
2.106a: X = Br, 78%
2.106b: X = H, 91%

2.107a: X= Br (65%)
2.107b: X= H (56%)

2.108a:X = Br (82%)
2.108b:X = H (81%)

Bisglycal *C*-aryl glycosides such as **2.112b** derived from naphthoquinone and 1,4-anthraquinone however cannot be prepared *via* the above-mentioned method directly. Those compounds were extremely sensitive to acid and selective hydrolysis of the glycal substituent *ortho* to the phenolic hydroxyl group occurred merely upon chromatography on silica or neutral alumina. This event necessitated the protection of one of the two hydroxyls as its silyl ether before the rearrangement to prevent the formation of acidic naphthol **2.112b**. An additional advantage of introducing a silyl protective group was that only the glycal geminal to the silyl-protected alcohol underwent 1,2-glycal rearrangement.[213]

Scheme 2.28



This regiospecific rearrangement is consistent with the mechanism proposed by Parker and discussed earlier.[215] Selectively chelation of the zinc chloride to the sterically more available hydroxyl group rather than the silyloxy group activates the hydroxyl and triggers the formation of the cyclohexadienyl cation. Migration of the glycal substituent geminal to the silyloxy group followed by loss of a proton, rather than the silyl moiety, resulted in the formation of aromatized product **2.112a** (Scheme 2.28). Therefore, it is possible to choose which one of two different glycal substituents becomes the migrating group by specifying the order of glycal introduction in the addition/silylation/addition/migration sequence. This methodology provides an alternate approach to bis-*C*-aryl glycosides, such as pluramycin and kidamycin antibiotics. To

date, no total syntheses of any naturally occurring bis-*C*-aryl glycosides have been reported.[217]

2.2.3 Transition Metal Mediated Coupling

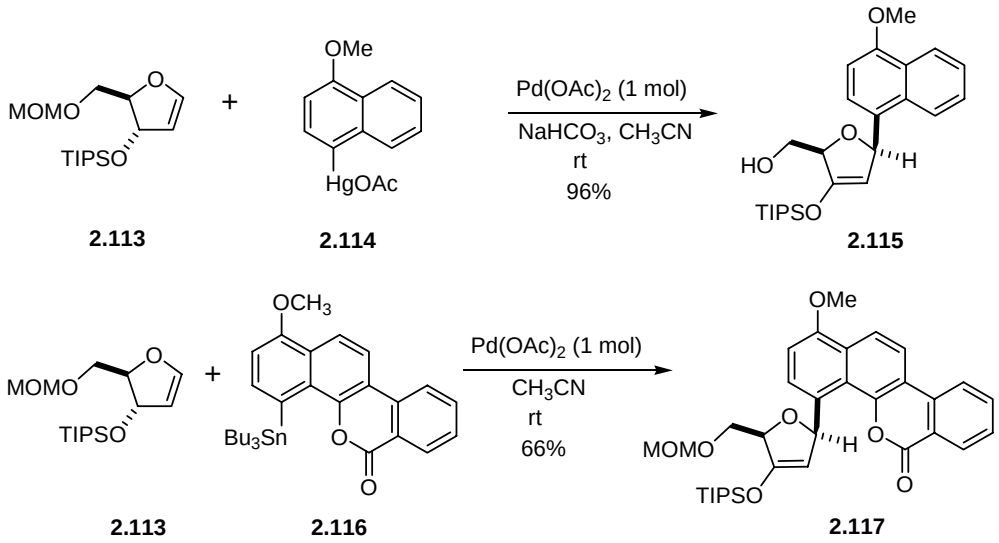
Although electrophilic and nucleophilic substitutions at the anomeric carbon have provided access to a variety of *C*-aryl glycosides, they have their own limitations. The use of strong Lewis acid in electrophilic sugar substitution and the strong basicity of anomeric organolithium in nucleophilic sugar substitution preclude the presence of functionalities that are acid or base sensitive. On the other hand, transition metal mediated cross coupling typically entails milder conditions and therefore has an advantage of broad functional group tolerance. Substantial progress has been made in the past decade in processes that rely on the cross coupling of appropriately functionalized aromatic compounds with carbohydrate derivatives. Couplings have been performed with aryl organometallic species and iodoglycals or alternatively with aryl halides and metallated glycals.

2.2.3.1 Heck Type Coupling

Daves was the one of first to apply a Heck-type coupling to prepare *C*-aryl glycosides. He observed that stirring a solution of 4-methoxynaphthyl-mercuric acetate **2.114,** which was prepared by electrophilic aromatic substitution using mercury acetate,[218] and glycal **2.113** with palladium acetate at room temperature afforded the *C*-aryl glycoside **2.115** in 96% yield.[219] The method was later extended to include arylstannanes such as **2.116** (Scheme 2.29).[219, 220] The regioselectivity was excellent with the tin or mercury directing the site of glycosidation. The double bond thus formed

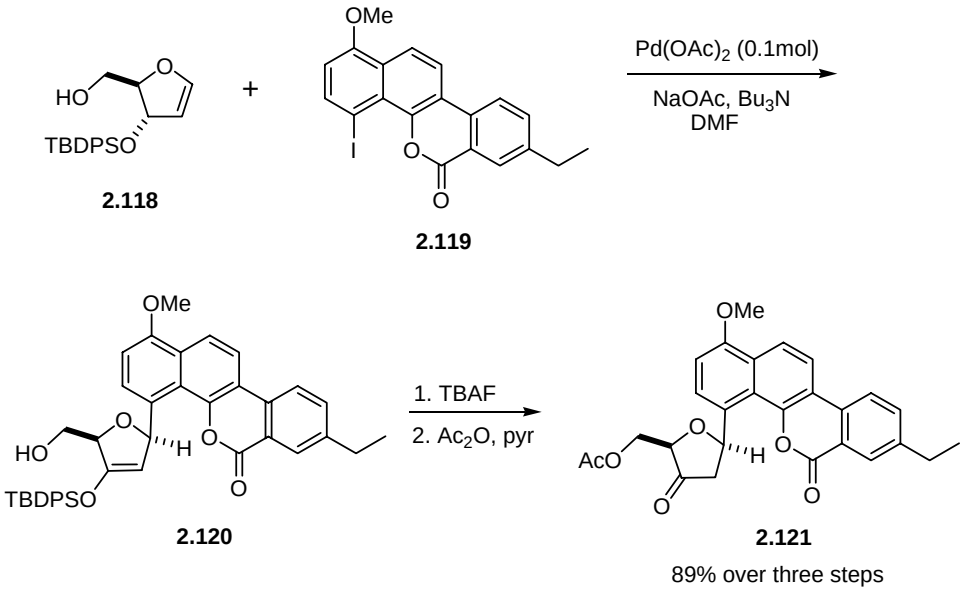
was well positioned for further manipulation. The main drawbacks of this approach to *C*-aryl glycosides were the mandatory use of stoichiometric palladium acetate and the formation of multiple products in some cases due to other competing decomposition pathways of the organopalladium intermediate.

Scheme 2.29



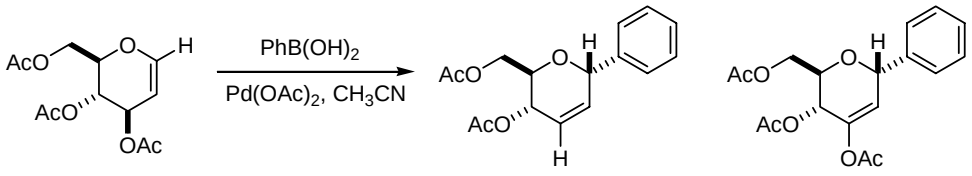
It was soon discovered that when an aryl iodide was used in place of the arylmercuric acetate, only a catalytic amount of palladium acetate was required.[221] The formation of the aryl palladium complex occurred in this case *via* oxidative addition of the aryl iodide to the palladium (0) catalyst. In a model study directed towards the synthesis of gilvocarcin by Daves and coworkers,[221] aryl iodide **2.119** was reacted with furanoglycal **2.118** to furnish *C*-aryl glycoside **2.120**. Desilylation with TBAF followed by acetylation delivered **2.121** in 89% yield over three steps (Scheme 2.30).

Scheme 2.30



Maddaford however observed exclusive formation of the Ferrier-type product **2.123** resulting from *anti* elimination of palladium acetate in the coupling of tri-*O*-acetyl glycal **2.122** with phenylboronic acid (Scheme 2.31).[222] The *anti* palladium acetate elimination pathway regenerated the active palladium catalyst at the end of the reaction, therefore only a catalytic amount of palladium was needed. The author suggested that the difference was caused by the reversible β-hydride elimination under the reaction condition.

Scheme 2.31



2.122 **2.123** 82% **2.124** 0%

More recently, Schmidt[223] reported a palladium (0)-catalyzed Heck arylation of substituted dihydropyran with aryldiazonium tetrafluoroborate. High C1, C5-*trans* selectivity was observed.

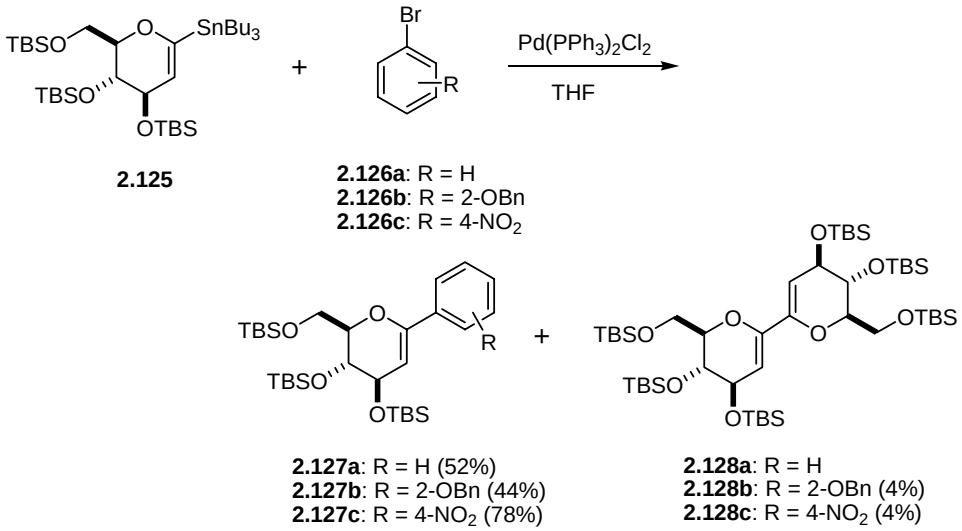
2.2.3.2 Stille/Negishi/Suzuki Type Coupling

It was later discovered that introduction of functional groups in both glycosyl donor and acceptor provided the mildest and the most efficient protocol for palladium-mediated arylation of glycals. The palladium promoter can be used substoichiometrically, and the *C*-aryl glycoside is formed through reductive elimination instead of β-hydride elimination. Since the glycal double bond doesn't participate in the coupling, no double bond isomer is observed,

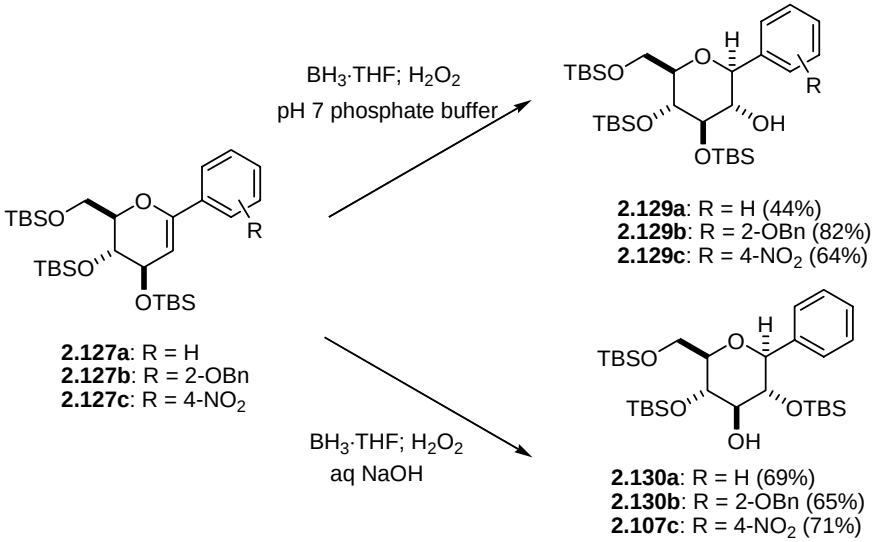
Friesen,[224] Beau[225] and Tius[226, 227] disclosed independently at roughly the same time that a Stille-type cross coupling of *C*-1 tributylstannyl glycal **2.125** with aryl bromides **2.126** in the presence of a palladium catalyst to give *C*-aryl glycosides **2.127**. Varying amounts of dimer **2.128** were isolated accounting for up to 30% of the staring material (Scheme 2.32). Aryl bromides with electron-withdrawing substituents generally provided higher yield of the coupled products than those with electron-donating substituents. The glycal double bond in the final product **2.127a-c** could be functionalized through regio- and stereo-selective hydroboration-oxidation to provide the 2-oxy sugar

derivatives **2.129a-c** or **2.130a-c** depending on the reaction conditions (Scheme 2.33).[225, 228]

Scheme 2.32

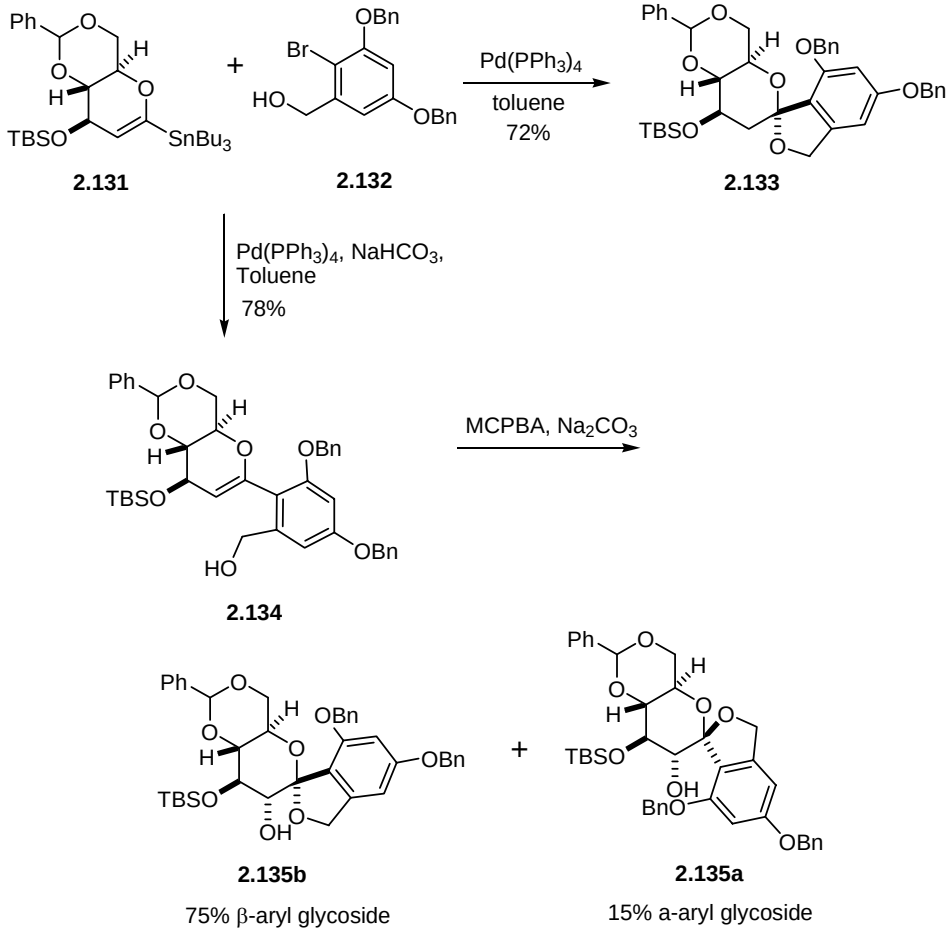


Scheme 2.33



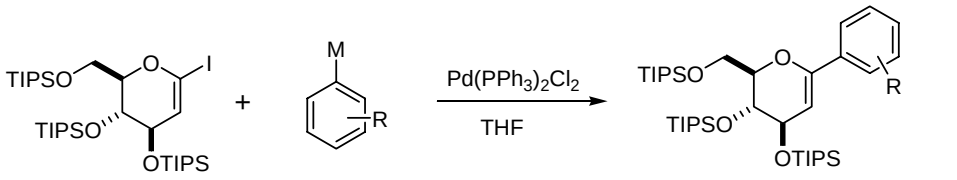
In Beau's syntheses of the core structures of papulacandins and chaetiacandin,[229] 1-stannylglycal **2.131** was allowed to react with aryl bromide **2.132** to provide the bicycloketal **2.133** as a single diastereomer at the spiro center. The formation of the tetracycle was explained by an acid catalyzed ketalization of the initial product **2.134**. Coupling under buffered conditions provided the expected *C*-aryl glycal **2.134** in 78% yield. Stereoselective epoxidation of **2.134** with *meta*-chloroperbenzoic acid at low temperature produced two anomeric isomers, β-*C*-aryl glycoside **2.135b** (70%) and α-*C*-aryl glycoside **2.135a** (15%). Both isomers were converted to the core structure of papulacandins (Scheme 2.34).

Scheme 2.34



Friesen later reversed the polarities of the glycosyl donor and acceptor.[230] He discovered that the reaction of iodoglycal **2.136** with phenylzinc chloride **2.137a** or phenylboronic acid **2.137b** in the presence of $(PPh_3)_2PdCl_2$ catalyst proceeded cleanly and gave high yields of coupling products **2.138a** and **2.138b**, respectively (Scheme 2.35). This modification delivered *C*-aryl glycosides in significantly higher yields, and the formation of glycal dimers was not observed even with electron-rich aromatic glycosyl acceptors.

Scheme 2.35



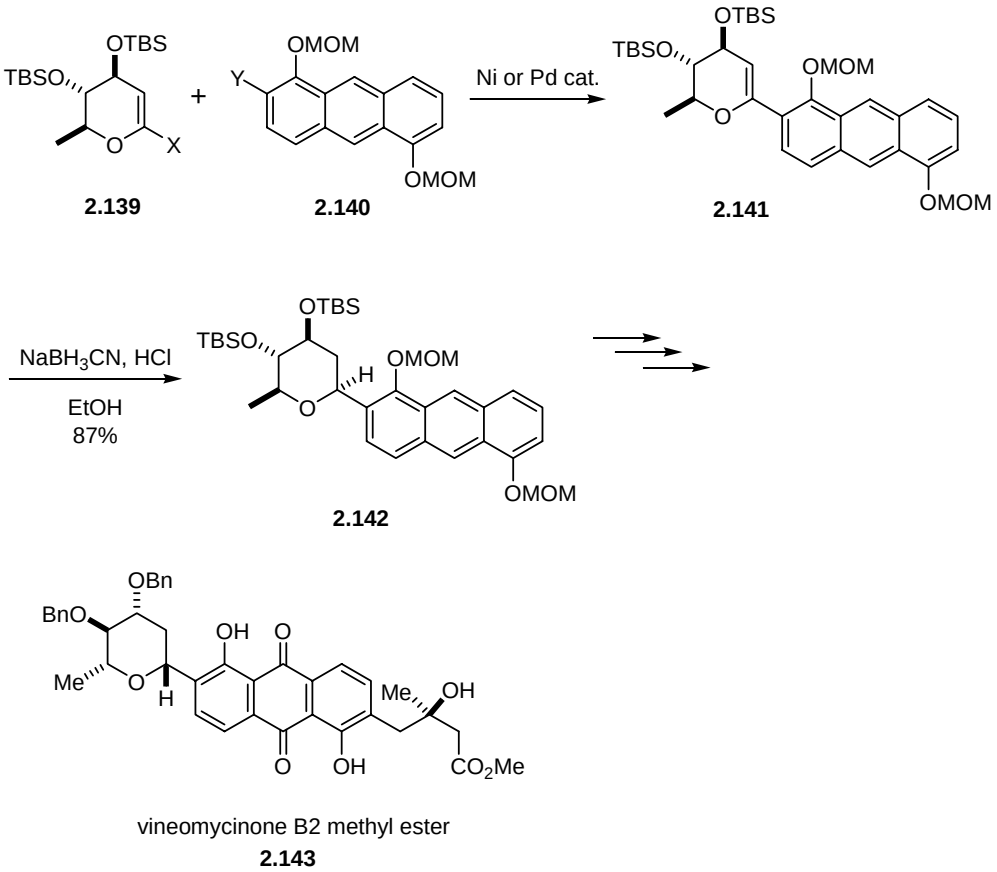
2.136

2.137a: R = H, M = ZnCl
2.137b: R = H, M = $B(OH)_2$
2.137c: R = H, M = $SnBu_3$

2.138a: R = H, M = ZnCl, 90%
2.138b: R = H, M = $B(OH)_2$, 81%
2.138c: R = H, M = $SnBu_3$, 20%

Tius explored both the Stille and the Negishi coupling in his total synthesis of vineomycinone B2 methyl ester.[226, 227] The couplings of glycal **2.139** and anthracene **2.140** in the presence of both Ni and Pd catalyst were examined. Reaction between metalloanthracene **2.140 (**Y = MgBr, ZnCl, $SnBu_3$) and iodo sugar **2.139** (X = I) gave **2.141** in yields no higher than 32%. A significantly improved yield of 79% was obtained with glycosylzinc chloride **2.139** (X = ZnCl) as nucleophilic glycosyl donor and aryl iodide **2.140 (**Y = I) as glycosyl acceptor. The optimal yield can only be obtained when the active Pd(0) catalyst was generated *in situ* through reduction of $Pd(PPh_3)_2C1_2$ with diisobutylaluminum hydride. Stereoselective reduction of *C*-anthracyl glycal **2.141** in acidic $NaBH_3CN$ produced the advanced synthetic intermediate **2.142** as a single diastereomer in 87% yield (Scheme 2.36).

Scheme 2.36



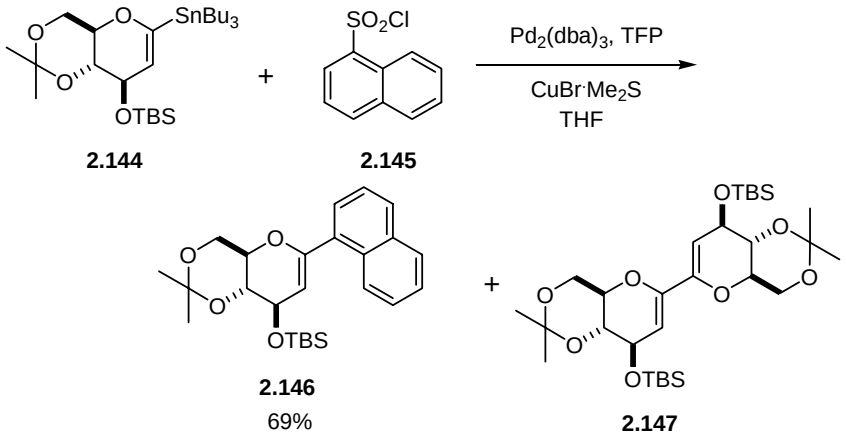
2.139 **2.140** **2.141**

2.142

vineomycinone B2 methyl ester
2.143

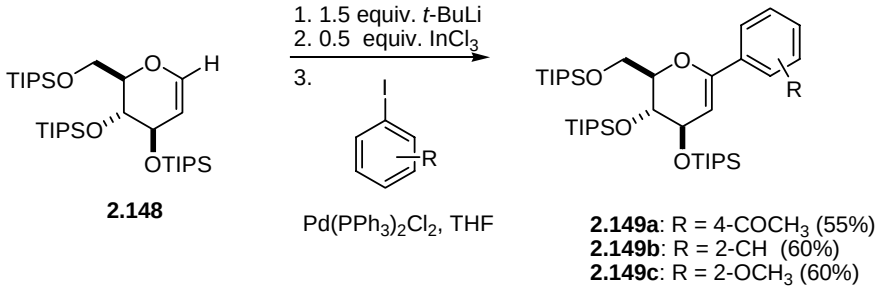
Recently, Vogel[231] demonstrated that the arylmethanesulfonyl chloride **2.145** and glycal stannane **2.144** underwent Stille cross coupling with concomitant desulfitation to afford **2.146** in the presence of $Pd_2(dba)_3$, trifurylphosphine (TFP), and $CuBr{\cdot}SMe_2$ (Scheme 2.37). The formation of glycal homocoupling dimer **2.147**, however, was not suppressed under the reaction conditions.

Scheme 2.37



Minehan[232] developed an approach to *C*-aryl glycoside involving coupling between aryl iodides and glycal indiums. Reactions with electron-deficient and electron-rich aryl iodides proceeded equally well affording the coupled product in moderate yields (Scheme 2.38). Aryl bromides typically gave inferior results. The exact nature of the organoindium species was not clear, and the author suggested either a mono- or di-glucal indium was formed during transmetallation.

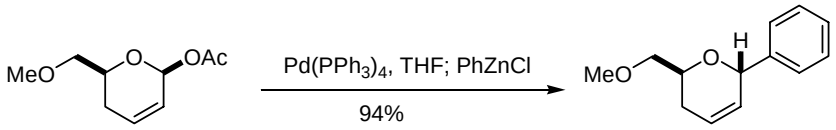
Scheme 2.38



2.2.3.3 Palladium π-Allyl Chemistry: Carbon-Ferrier Reaction

Dunkerton[233] pioneered the use of π-allylpalladium complex in the synthesis of *C*-glycosides. Treatment of **2.150** with phenylzinc chloride in the presence of 5 mol% of $Pd(PPh_3)_4$ gave the *C*-glycoside **2.151** in 94% yield (Scheme 2.39). This type of reaction was regio- and stereoselective with inversion of the stereochemistry at anomeric center. This stereochemical outcome was consistent with inversion during the formation of the π-allyl-palladium complex followed by retention of configuration in the transmetallation and reductive elimination steps. Substrates with C4-oxygenated groups gave at best moderate yields (16-30%) and the allylic transposed acetates didn't undergo alkylation under reaction conditions.

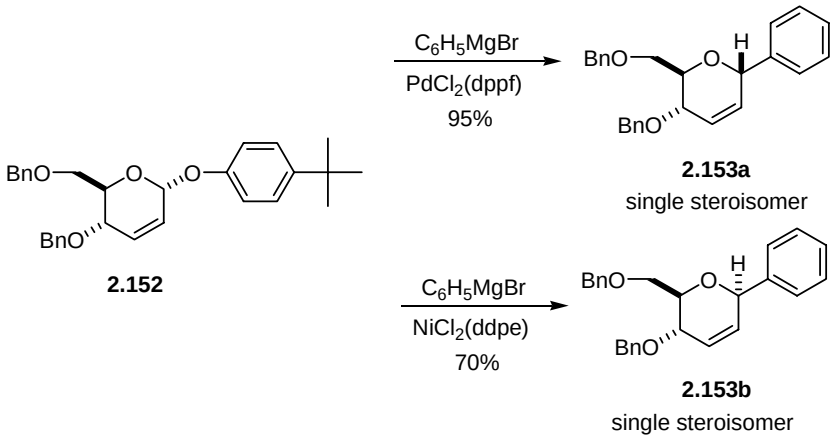
Scheme 2.39



2.150 **2.151**

Sinou and coworkers[234, 235] disclosed that treatment of 1-(4'-*tert*-butyl)phenyloxy-2-enopyranoside **2.152** with arylmagnesium bromide in the presence of catalytic $PdCl_2$(dppf) afforded the corresponding unsaturated *C*-aryl glycoside **2.153a** in excellent yield with retention of anomeric stereochemistry. When the same reaction was performed with $NiCl_2$(dppe) as the catalyst at -40 °C, the *C*-aryl glycoside **2.153b** with inverted stereochemistry at the anomeric carbon was obtained instead (Scheme 2.40). Other arylmetallic reagents, such as tetraphenylborate, phenylzinc chloride and tributylphenylstanne, gave inferior results.

Scheme 2.40



The author rationalized the reversed selectivity based on the assumption that oxidative addition of the Pd(0) or Ni(0) species to the unsaturated carbohydrate occurred with inversion of configuration at the anomeric center to give π-allyl intermediate. In the case of the allyl-Ni intermediate, transmetallation with the organomagnesium was followed by *syn* reductive elimination to form the unsaturated *C*-aryl glycoside with overall inversion of configuration. In the case of the allyl-Pd intermediate, *exo* attack of the organomagnesium reagent on the π-allyl intermediate delivered the substituted product with an overall retention of configuration resulting from double inversion.[236]

2.2.4 Pericyclic Reactions

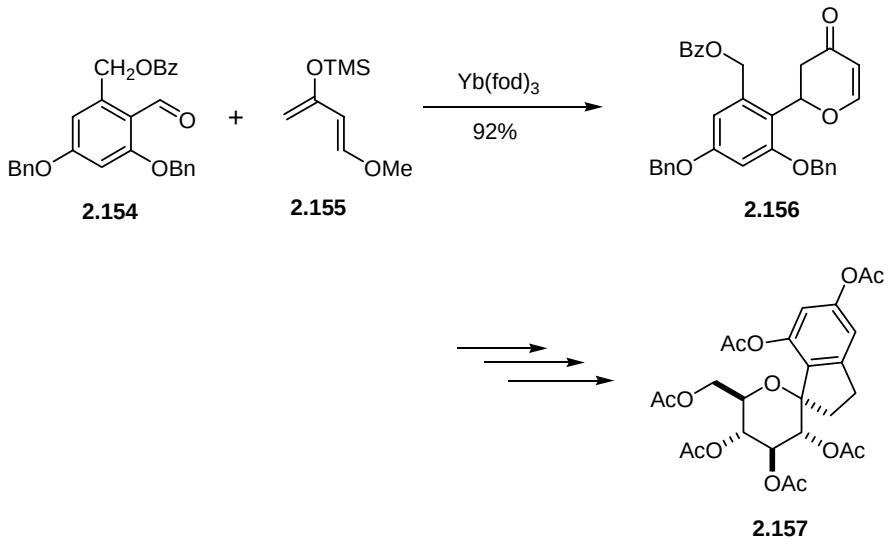
2.2.4.1 Diels-Alder Cycloaddition: De Novo Synthesis of Carbohydrate

The hetero Diels-Alder (HDA) reaction to assemble the dihydropyran skeleton has found application in *C*-aryl glycoside chemistry and represents probably the most powerful and widely used tool for *de novo* construction of carbohydrates on pre-existing

aromatic systems. The method is particularly useful for assembling non-natural sugars, which otherwise are difficult to prepare.

One of the early examples is found in Danishefsky's synthesis of the core structure of the papulacandins.[237] The ytterbium catalyzed, hetero Diels-Alder reaction of Danishefsky's diene **2.155** and aromatic aldehyde dieneophile **2.154** provided the substituted carbohydrate **2.156** (Scheme 2.41), which possessed proper functionality allowing further elaboration to papulacandins core.

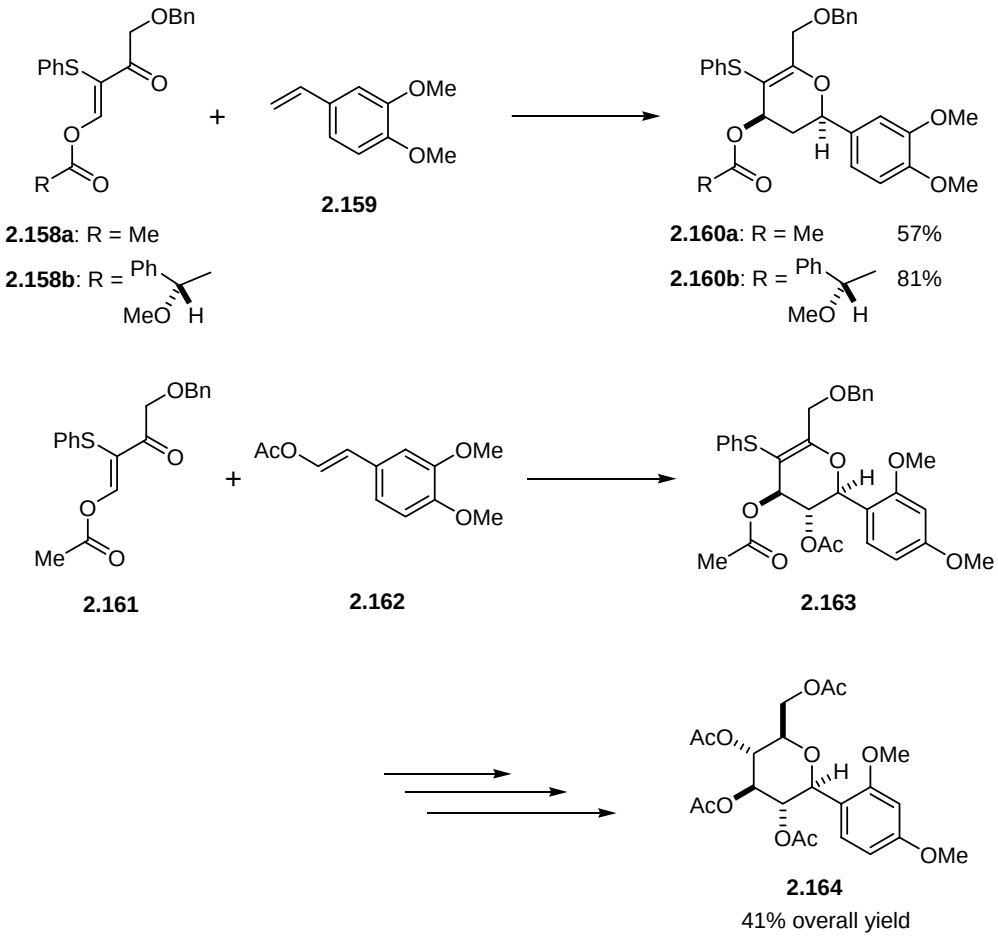
Scheme 2.41



Schmidt[238] reported the application of inverse electron demand hetero-Diels-Alder reactions for the synthesis of *C*-aryl glycosides. For example, the functionally substituted α,β-unsaturated carbonyl compounds **2.158a** and **2.158b** cyclized with substituted styrene **2.159** to yield *C*-aryl glycosides. The reaction, proceeded smoothly under high pressure (5.2 kbar, 60°C), in a regio- and *endo*-specifically fashion to deliver compounds **2.160a** and **2.160b** in 57 % and 81% yields, respectively. Subsequent desulfurization and hydroboration/oxidation delivered 2-deoxy-*C*-aryl glycosides.

Asymmetric induction of these reactions was illustrated using a *O*-methylmandeloyl group as a chiral auxiliary in the synthesis of **2.160b**, thus providing convenient entries into both D- and L-sugars. Schmidt also obtained 2-oxy-*C*-aryl glycoside **2.164** in 41% overall yield after four steps using β-acetoxy styrene **2.162** as the dienophile (Scheme 2.42). The use of an acetoxy group was crucial to the success of the cycloaddition, and the author noted that the β-alkoxy styrene gave the opposite regioselectivity.

Scheme 2.42



Yamamoto[239] developed the first reliable asymmetric hetero-Diels-Alder approach to *C*-aryl glycosides. The chiral BINOL-AlMe complex **2.165** was found to be highly efficient for HDA reaction of various aldehydes and Danishefsky type dienes. The presence of a bulky triarylsilyl moiety was crucial for obtaining high enantioface differentiation of the prochiral aldehydes. Other chiral Lewis acids have also been successful used in the HAD reaction, among them are Yamamoto's chiral (acyloxy)borane (CAB) catalyst **2.166**,[240, 241] Jacobsen's tridentate Schiff base chromium(III) complexes **2.167**,[242] Hashimoto's chiral dirhodium(ii) carboxylate and carboxamidate catalysts,[243] Ding's BINOLate-zinc complex **2.168**,[244] Jorgensen's chiral C2-symmetric BOX-copper (II) catalyst[245] (Figure 2.4).

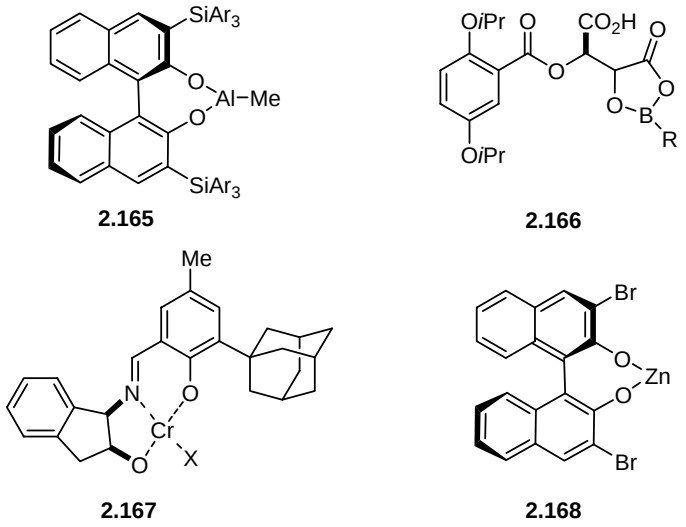


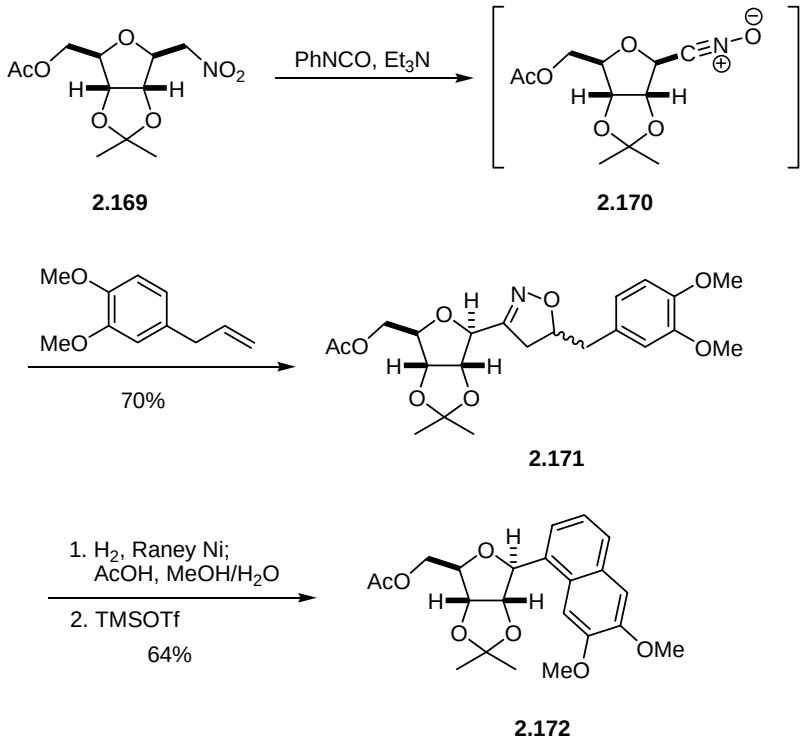
Figure 2.4: Chiral Lewis acid used in HDA reactions

2.2.4.2 [2+3] Dipolar Cycloaddition

[2+3] cycloadditions have been used to append functional groups on an already existing carbohydrate or to construct the aromatic appendage. Kozikowski[246] used a

nitrile oxide dipolar cycloaddition to build the aromatic attachment (Scheme 2.45). The *C*-1 nitromethyl substituted carbohydrate derivative **2.169** was exposed to phenyl isocyanate and triethylamine to generate nitrile oxide **2.170** *in situ*, which was reacted with a allylated arene to provide isoxazoline **2.171**. Hydrogenation using Raney nickel cleaved N-O bond providing an intermediate β-hydroxyketone that was readily cyclized in the presence of TMSOTf to deliver *C*-naphthyl glycoside **2.172** (Scheme 2.43).

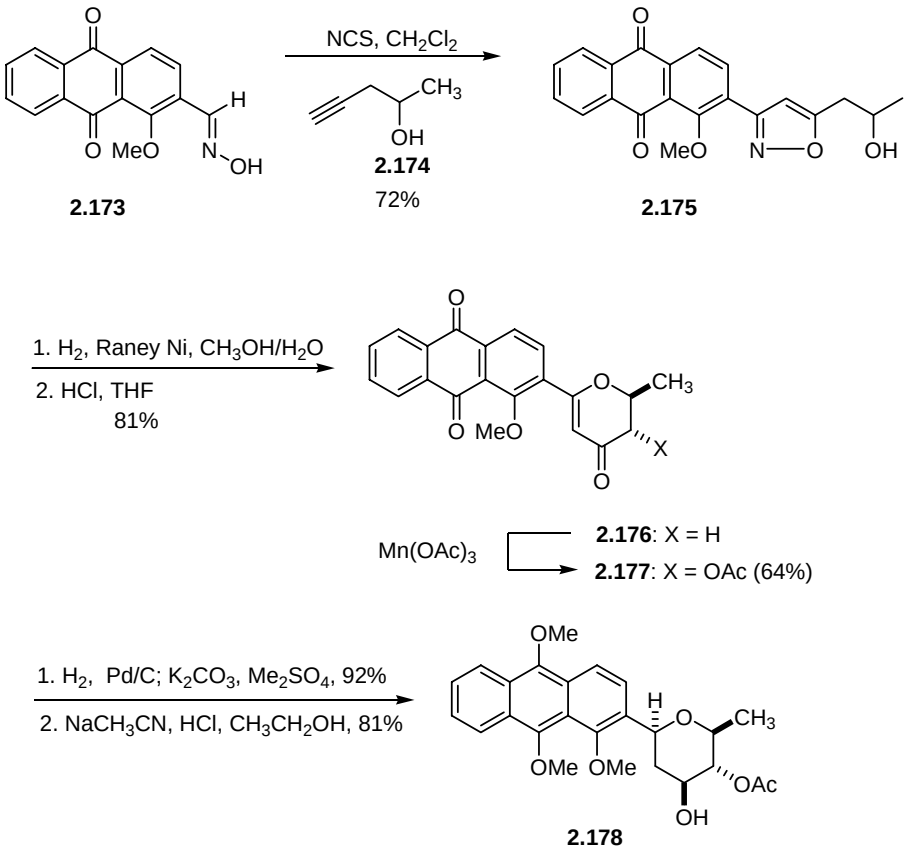
Scheme 2.43



In contrast, Hauser[247] constructed the carbohydrate backbone from a cycloadduct obtained from a dipolar cycloaddition. The nitrile oxide, which was generated from reaction of the anthraquinone oxime **2.173** with NCS, underwent cycloaddition with racemic **2.174** to afford the isoxazole **2.175**. Hydrogenolysis of **2.175** followed by acid

treatment to effect cyclization/dehydration of the intermediate diketone afforded pyranone **2.176**. α-Acetoxylation of **2.176** with manganic acetate gave a mixture (3:1) of C2-epimers **2.177**. The major isomer was reduced under Tius' conditions[227] to deliver 2-deoxy-*C*-anthracyl glycoside **2.178** in 81% yield as a single diastereomer (Scheme 2.44).

Scheme 2.44



2.2.5 Intramolecular Ring Closure

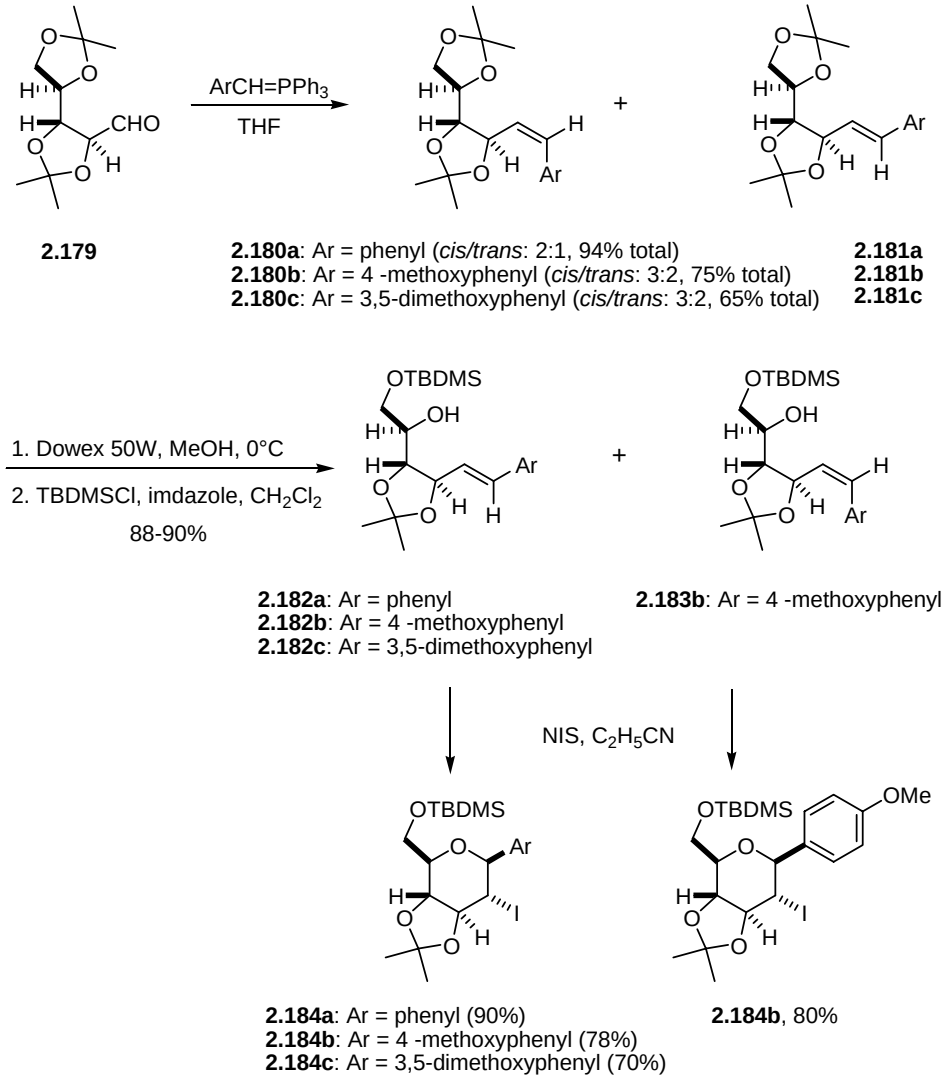
Intramolecular cyclization consists of a broad category of reactions where either the *C*-aryl substituent or the sugar backbone can be built on existing structures. These cyclizations range from simple electrophilic addition and nucleophilic substitution to

metal-catalyzed alkyne trimerization and ring closing metathesis. Biomimetic synthesis involving cascade aldol condensation will also be covered in this section.

2.2.5.1 Olefination/Cyclization Sequence

Schmidt[248] converted aldehydo-D-arabinose **2.179** into 2-deoxy-*C*-aryl glucopyranosides by iodonium-induced cyclization of **2.182** and **2.183**. Both geometric isomers cyclized if an electron-donating methoxy group present on the *para*-position of the aromatic ring, although the *trans*-double bond isomer cyclized at a significantly greater rate. The cyclization gave exclusively β-anomers, regardless of the double bond geometry in the starting material (Scheme 2.45). When an electron-withdrawing nitro group present, no cyclization was observed with either double bond isomer. The stereochemical outcome was rationalized by proposing a reversible electrophilic attack of *N*-iodo succinamide on the olefin double bond forming a three-membered iodonium ion followed by C5-hydroxyl attack at the C1 carbon. Therefore the presence of electron-withdrawing group was deleterious to the cyclization. Reversible NIS attack at the double bond does not result in ring closure in the *cis* isomers due to the unfavorable 1,3-interaction in the chair-like transition state. However, in the more electron-rich **2.183b**, this problem was overcome by generating a more stable *para*-methoxybenzyl cation followed by rotation of C1-C2 bond leading to the thermodynamically stable iodonium ion.[248]

Scheme 2.45



2.179

2.180a: Ar = phenyl (*cis/trans*: 2:1, 94% total) **2.181a**
2.180b: Ar = 4 -methoxyphenyl (*cis/trans*: 3:2, 75% total) **2.181b**
2.180c: Ar = 3,5-dimethoxyphenyl (*cis/trans*: 3:2, 65% total) **2.181c**

2.182a: Ar = phenyl
2.182b: Ar = 4 -methoxyphenyl
2.182c: Ar = 3,5-dimethoxyphenyl

2.183b: Ar = 4 -methoxyphenyl

2.184a: Ar = phenyl (90%)
2.184b: Ar = 4 -methoxyphenyl (78%)
2.184c: Ar = 3,5-dimethoxyphenyl (70%)

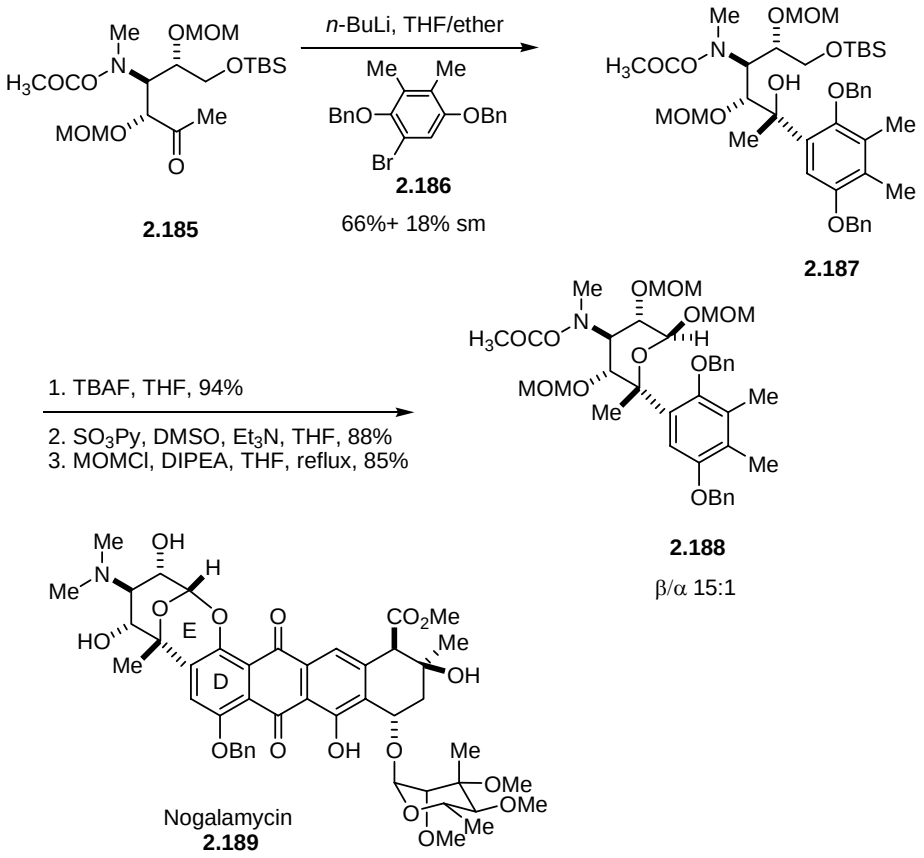
2.184b, 80%

2.2.5.2 Ketalization and Etherification

Terashima, in the synthesis of nogalamycin congener,[249-251] used a ketalization to form the tetrahydropyran ring. The aryl substitution was installed though a nucleophilic addition of an aryllithium to D-arabinose derived ketone **2.185**. Removal of the silyl protective group in **2.187** followed by regioselective oxidation of the resultant

1,5-diol triggered a spontaneous cyclization to form a mixture of epimeric acetals, which was immediately alkylated with chloromethyl methyl ether to deliver **2.188** (Scheme 2.46). Hydrogenolysis followed by Lewis acid catalyzed transacetalization then delivered the DEF ring system of nogalamycin (**2.189**).

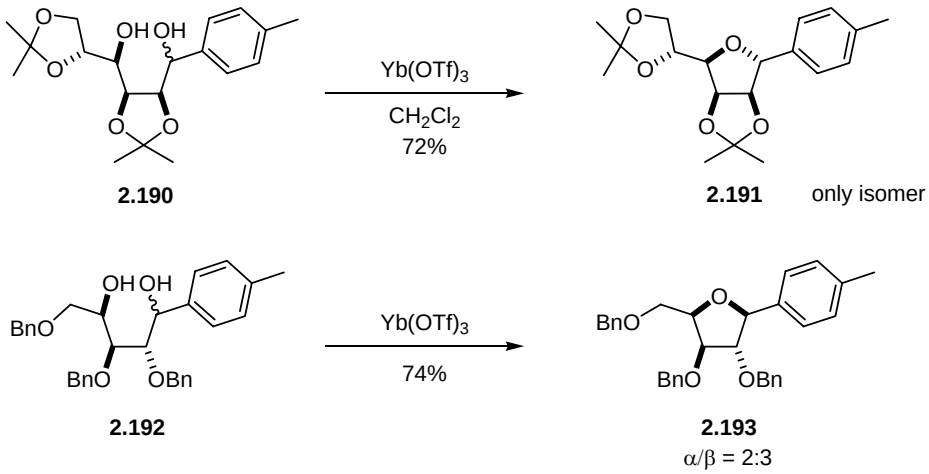
Scheme 2.46



Cyclodehydration of 1,4-diols under either Bronsted acidic or Lewis acidic conditions were used to synthesis *C*-aryl glycofuranosides. The requisite 1,4 diol was generally obtained by nucleophilic addition of an organometallic reagent to a sugar furanose. Sharma and co-workers discovered that *C*-phenyl glycoside **2.191** and **2.192** could be obtained in good yields by simply stirring a solution of 1,4-diol **2.190** or **2.192**

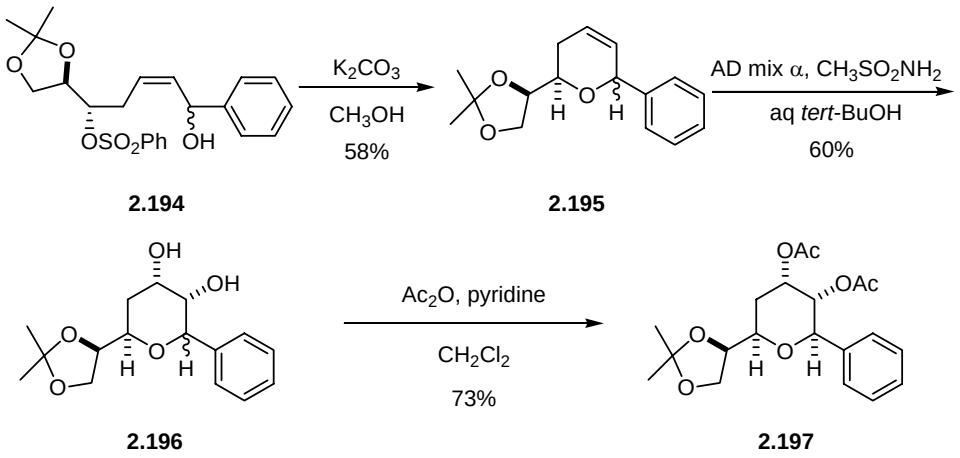
with a catalytic amount of $Yb(OTf)_3$, respectively (Scheme 2.47).[252] The stereoselectivity was substrate dependent and not always predictable; both high α and β selectivities were sometimes observed.

Scheme 2.47



Nucleophilic substitution was also used to access *C*-aryl glycoside. For example, displacement of the C5-sulfonate in **2.194** furnished dihydropyran **2.195** as an inseparable diastereomeric mixture,[253] which was subjected asymmetric dihydroxylation and subsequent acetylation to afford a single β-aryl glycoside **2.197** (Scheme 2.48). The author suggested that anomerization probably occurred during acetylation. Krishna also used a Mitsunobu etherification to activate 1,4-diol in order to graft a tetrahydropyran carbohydrate onto a pre-existing aryl group.[254]

Scheme 2.48

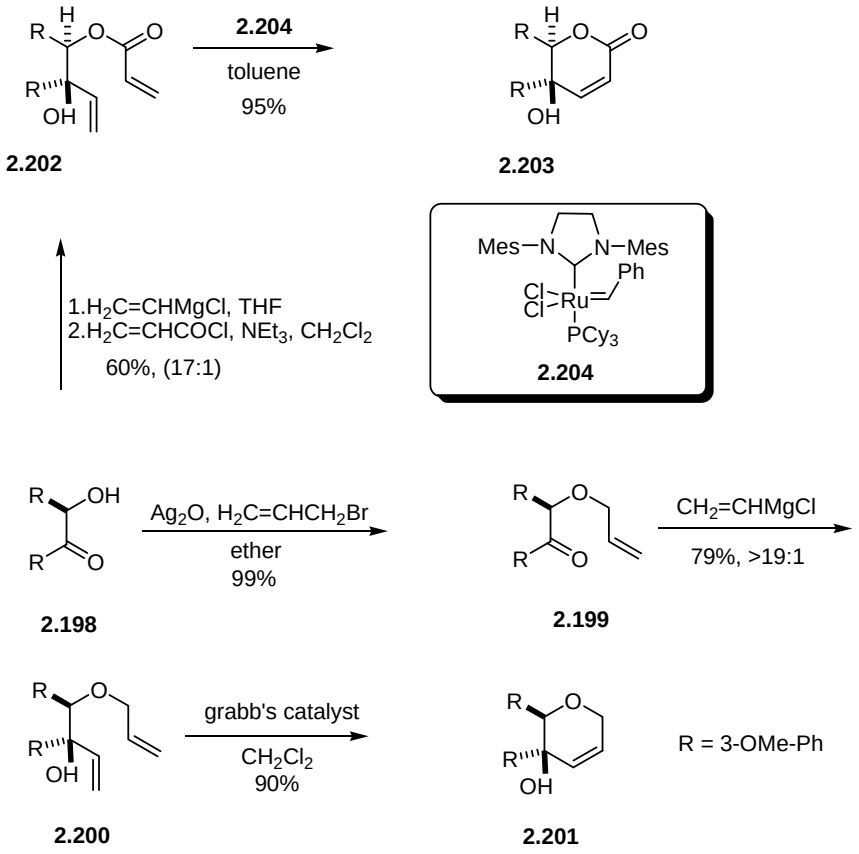


2.2.5.3 Ring Closing Metathesis

Schmidt, in the late nineties, explored the possibility of ring closing metathesis (RCM) for the synthesis of *C*-aryl glycoside.[255-263] He prepared a variety of 2-aryl dihydropyrans *via* RCM. In one of his recent publications, Schmidt obtained *C*-aryl glycoside **2.201** as a single diastereomer in enantiomerically pure form.[263] Treatment of optically pure α-hydroxy ketone **2.198** with allyl bromide and silver oxide afforded *R*-allyloxy ketone **2.199** without any noticeable racemization. Reaction of **2.199** with vinylmagnesium chloride produced the requisite diene **2.200** with high diastereoselectivity. Ring closing metathesis of the allylic homoallylic ethers in the presence of 3 mol % of the first generation Grubbs' catalyst gave the dihydropyran **2.201** in excellent yields. Slight modification of reaction sequence allowed the ready conversion of *R*-hydroxy ketone **2.198** to α,β-unsaturated lactones **2.202**. Ring-closing metathesis of diene **2.202** delivered dihydropyranone **2.203** in excellent yield with second-generation Grubbs' catalyst (Scheme 2.49). The dihydropyrans could be readily converted to

carbohydrates through dihydroxylation, stereoselective hydride reduction or epoxidation.[256]

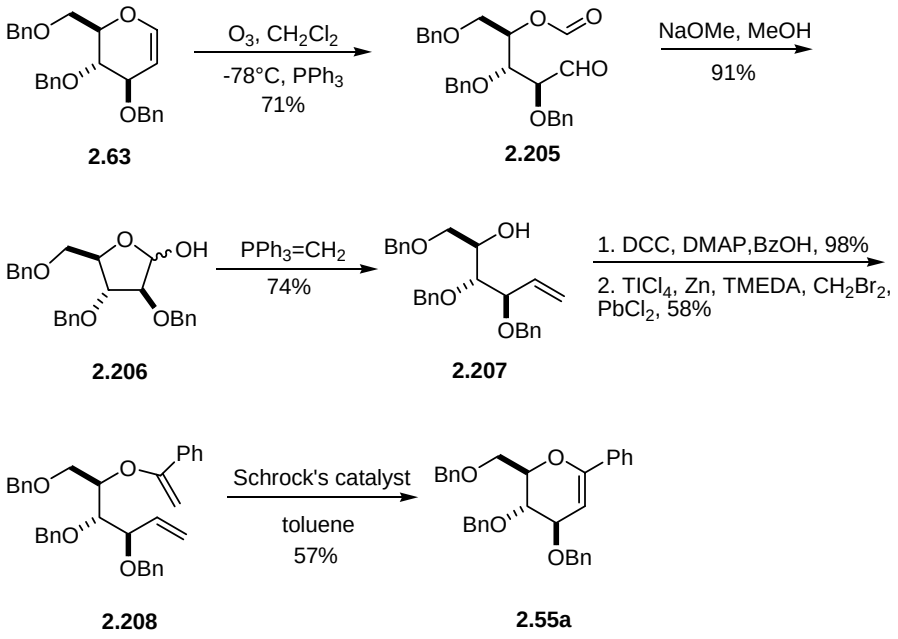
Scheme 2.49



Postema also developed an approach to *C*-aryl glycal using ring closing metathesis.[264] For example, Wittig olefination of pentose **2.206** afforded alcohol **2.207**, which was acylated with the appropriate carboxylic acid. Subsequent olefination afforded the metathesis precursor **2.208**. Exposing diene **2.208** to Schrock's catalyst afforded *C*-aryl glycal **2.55a** in moderate yield (Scheme 2.50). The method provides a complementary approach to C2-aryl substituted dihydropyrans. However this approach

lacks practicability due to its length and high catalyst loading (25 to 50 mol%) in order to obtain synthetically useful yields.[264]

Scheme 2.50

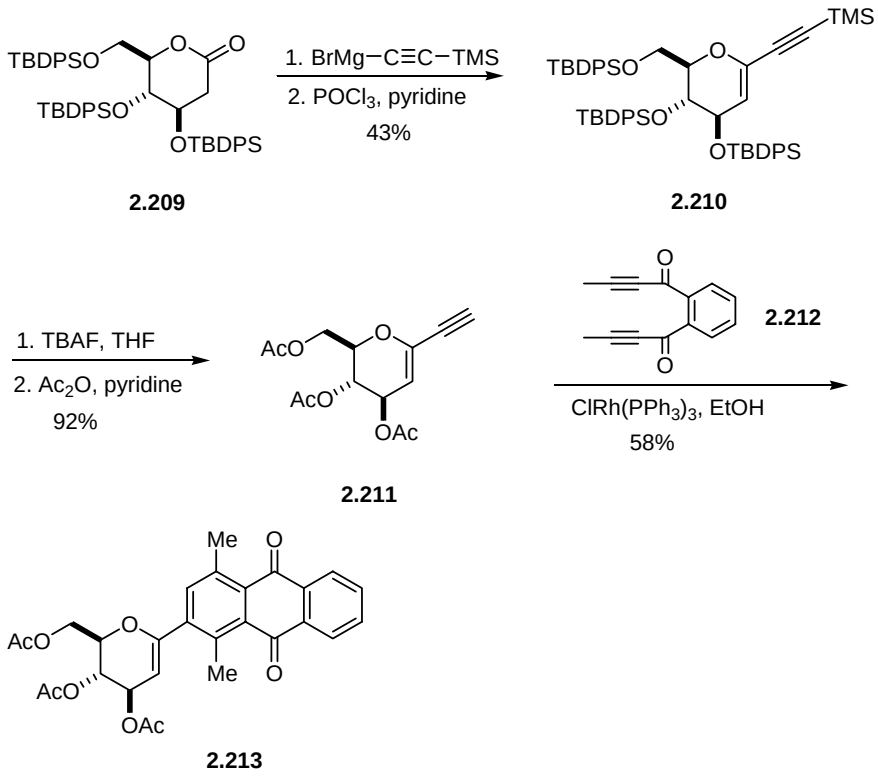


2.2.5.4 The Metal-Catalyzed Cyclization

One route to *C*-aryl glycosides involves the construction of the aryl group on a pre-existing carbohydrate framework. Using this strategy, McDonald[265] was able to access the core structures of papulacandins and vineomycinone using rhodium catalyzed alkyne cyclotrimerization. The nucleophilic addition of 2-(trimethylsilyl)ethynylmagnesium bromide to the lactone **2.209** followed by dehydration of the intermediate hemiacetal yielded the alkynylglycal **2.210**. Fluoride induced deprotection of the silyl ether followed by reprotection of the alcohols as their acetates furnished enyne **2.211**. Cyclotrimerization with diyne **2.212** in the presence of 20 mol% Wilkinson's catalyst in ethanol delivered glycosidyl anthraquinone **2.213** in moderate

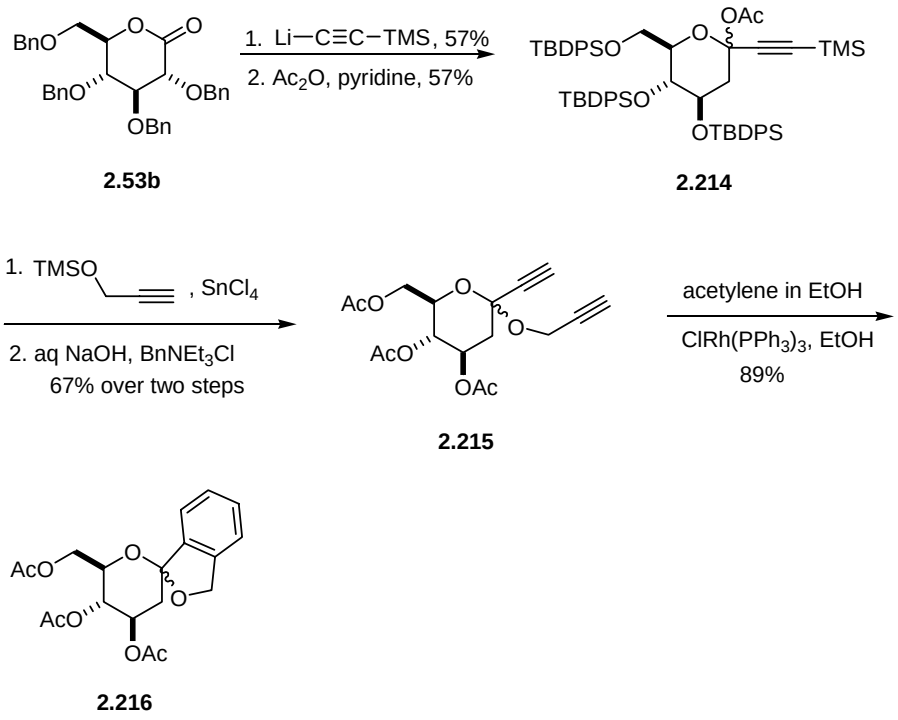
yield (Scheme 2.51). A lower yield of 35% was obtained when the carbohydrate protective groups remained *tert*-butyldiphenylsilyl ethers.

Scheme 2.51



McDonald also prepared spiroglycoside **2.216** using a similar strategy.[265] Addition of 2-(trimethylsilyl)ethynyllithium to gluconolactone **2.53b** followed by acetylation gave a mixture of anomeric acetates **2.214**. Lewis acid-catalyzed glycosidation afforded diyne **2.215**, and subsequent cyclotrimerization of each anomer in a saturated ethanolic solution of acetylene provided the corresponding unfunctionalized spirocyclic *C*-aryl glycosides **2.216** in excellent yield, which resembled the skeleton of papulacandins (Scheme 2.52).

Scheme 2.52



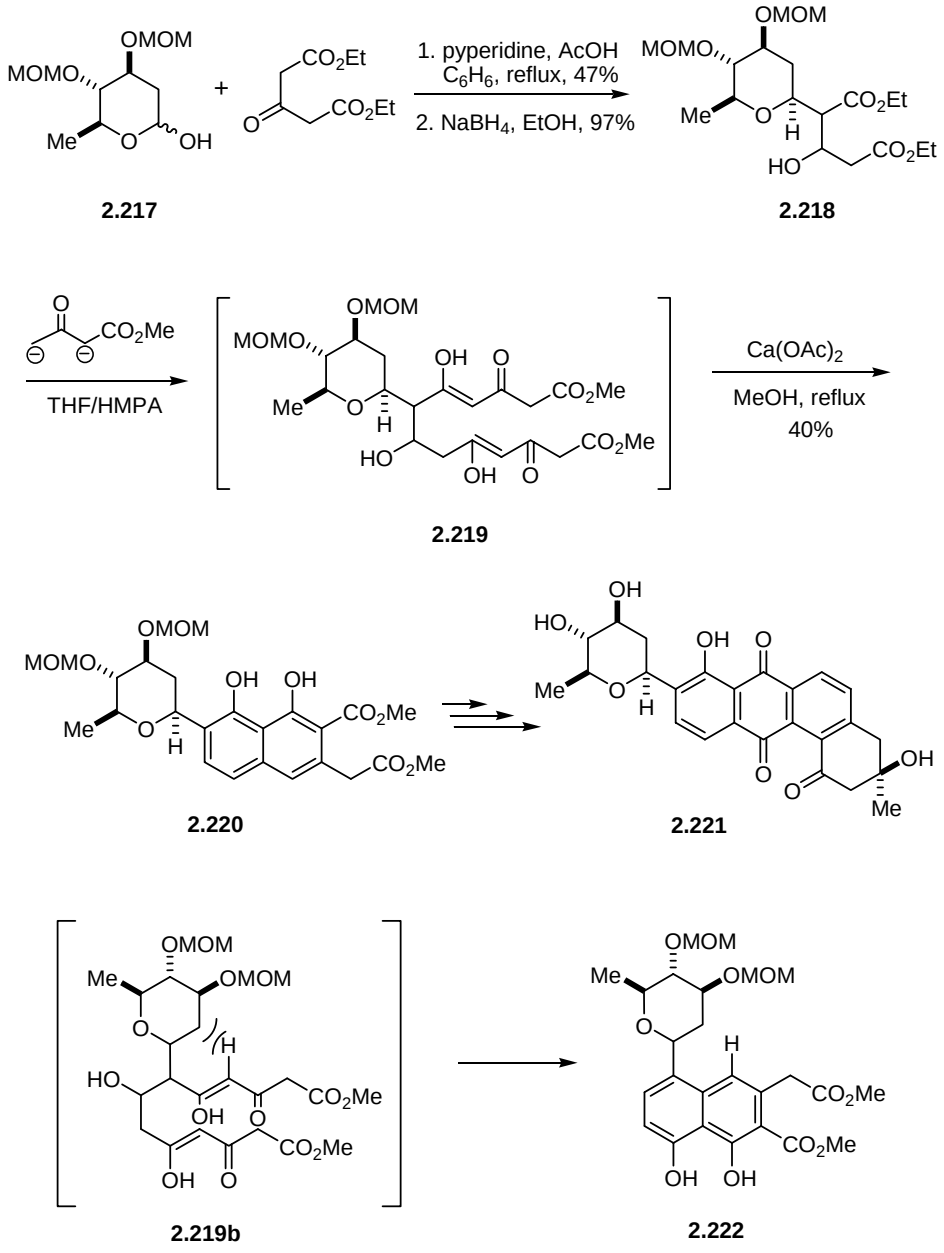
2.53b **2.214** **2.215** **2.216**

2.2.5.5 Biomimetic Synthesis

The major classes of *C*-aryl glycosides are believed to arise biosynthetically from polyketide precursors. A biomimetic synthesis of (-)-urdamycinone B utilizing cyclization of a previously formed *C*-glycosyl polyketide as key step was accomplished by Yamaguchi and coworkers in 1992.[266, 267] The 2-deoxy sugar **2.217**, which was readily prepared from L-rhamnal, was condensed with 3-oxoglutarate under Knoevenagel conditions followed by reduction with ethanolic $NaBH_4$ to afford **2.218**. Claisen condensation of **2.218** with the dianion of acetoacetate afforded **2.219**, which was cyclized *in situ* by treatment with $Ca(OAc)_2$ to produce the *C*-naphthyl glycosidic **2.220** as a single stereoisomer. No Group I *C*-aryl glycoside **2.222** was detected. The author explained the lack of conformer **2.219b**, the immediate precursor for **2.222** based on

unfavorable 1,3-allylic strain between the vinylic hydrogen and carbohydrate substituent. *C*-Naphthyl glycoside **2.220** was then converted into (-)-urdamycinone B, the enantiomer of the natural product (Scheme 2.53).

Scheme 2.53



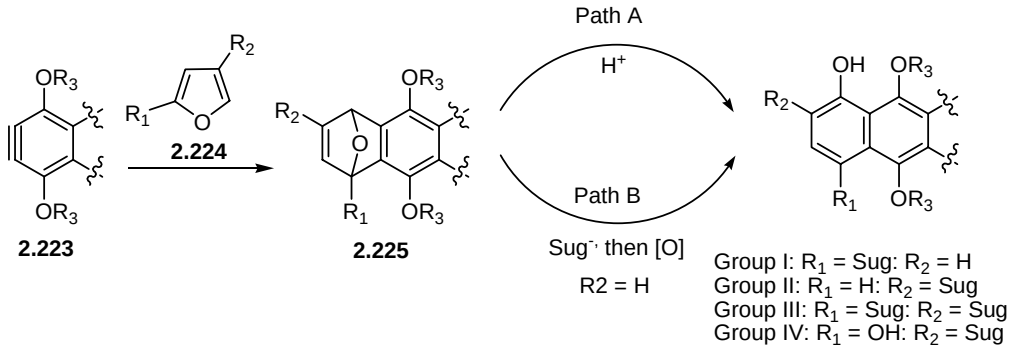
2.3 PRIOR WORK IN MARTIN GROUP

The previous sections have mainly focused on the synthesis of *C*-aryl glycoside on a case-by-case basis, consequently the importance of the control of the absolute stereochemistry at the anomeric center often appears to outweigh that of the control of regiochemistry, namely the placement of the carbohydrate moiety on the aromatic ring. However, in a systematic and unified approach to major classes of the *C*-aryl glycosides, the ability to deploy the carbohydrate moiety at a fixed position of an oxygenated aromatic core in a predictable manner and to preserve or invert the stereochemistry at the attachment in the subsequent transformations is not only important, it is required for such a method to be useful. In this area of chemistry, Suzuki and Parker have done tremendous amount of work. The major part of their work has already been covered in the previous sections. In this section, we will briefly review the work in the Martin group in developing a unified approach to major classes of *C*-aryl glycoside natural products based on glycosyl-furan/benzyne cycloadditions.[268, 269]

2.3.1 United Strategy For the Synthesis of C-Aryl Glycoside

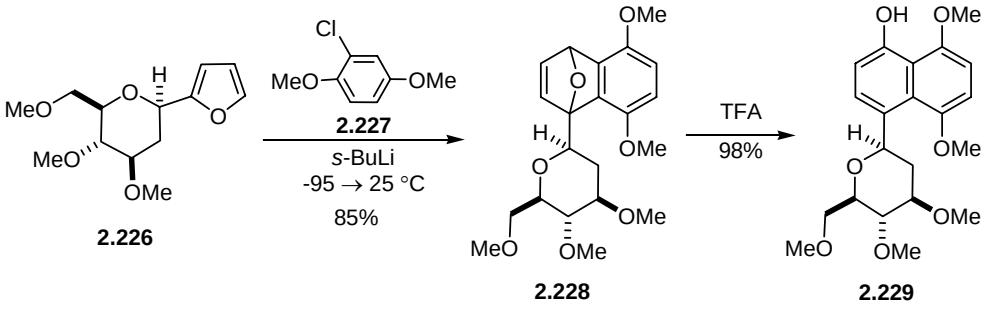
The strategy to access the four major structural types of *C*-aryl glycosides relies on the regiospecific opening of glycosyl furan/benzyne cycloadducts of the general type **2.225** as outlined in Scheme 2.54. The first route (Path A) features the cycloaddition of a glycosyl furan **2.224** (R_1 and/or R_2 = Sug) with a substituted benzyne **2.223** followed by acid-catalyzed opening of the resultant adduct **2.225** to give Group I–IV of *C*-aryl glycosides. The second pathway (Path B) involves the S_N2'-like opening of cycloadduct **2.225** (R_1 = H, Sug; R_2 = H) with sugar nucleophiles followed by oxidative aromatization to generate *C*-aryl glycosides of Group II, III and IV.

Scheme 2.54



The first approach is illustrated by deprotonation of **2.227** to give an anion that was allowed to warm to room temperature in the presence of 2-glycosyl furan **2.226**. During the course of warming, benzyne generation and cycloaddition ensued to give **2.228**. Acid-catalyzed rearrangement of **2.228** delivered the Group I *C*-aryl glycoside representative **2.229** as a single diastereomer in excellent overall yield (Scheme 2.55).

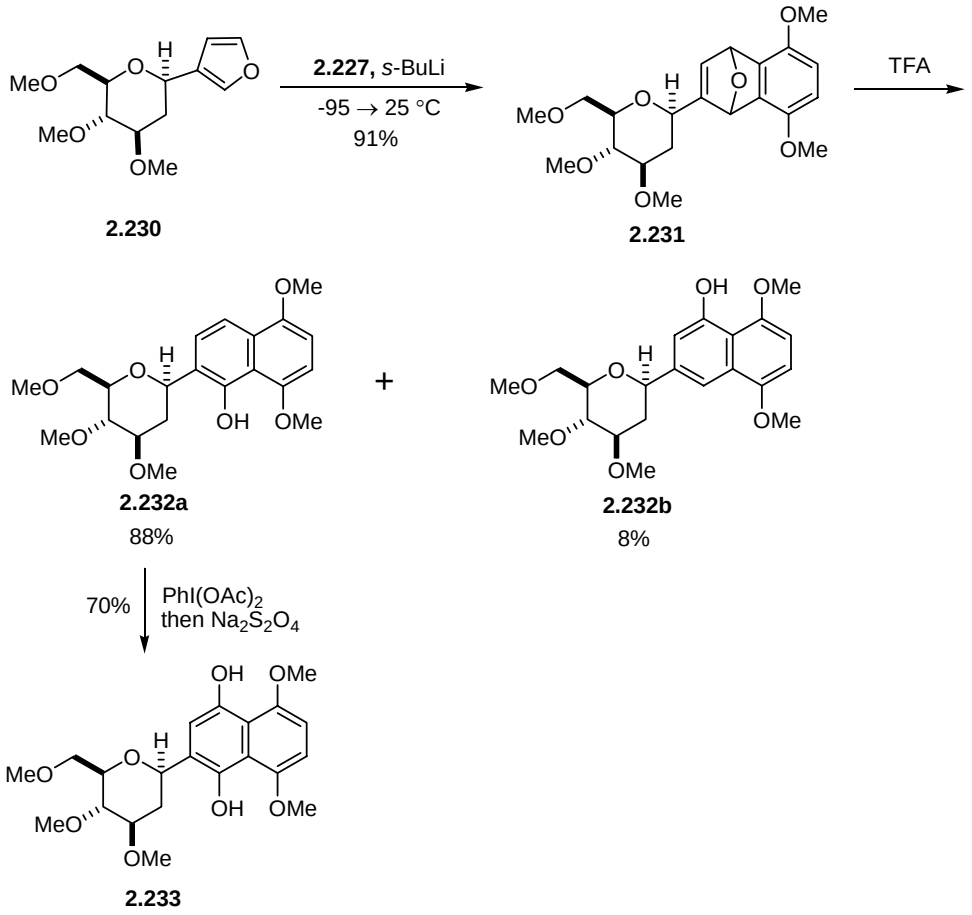
Scheme 2.55



In a relates sequence, cyclization of 3-glycosyl furan **2.230** and 1,4-dimethoxybenzyne followed by acid-catalyzed rearrangement gave the Group II *C*-aryl glycoside model **2.232a** in 80% yield as a single diastereomer accompanied with small amount (8%) of the isomeric *m*-substituted product **2.232b**. Oxidation of **2.232a** with

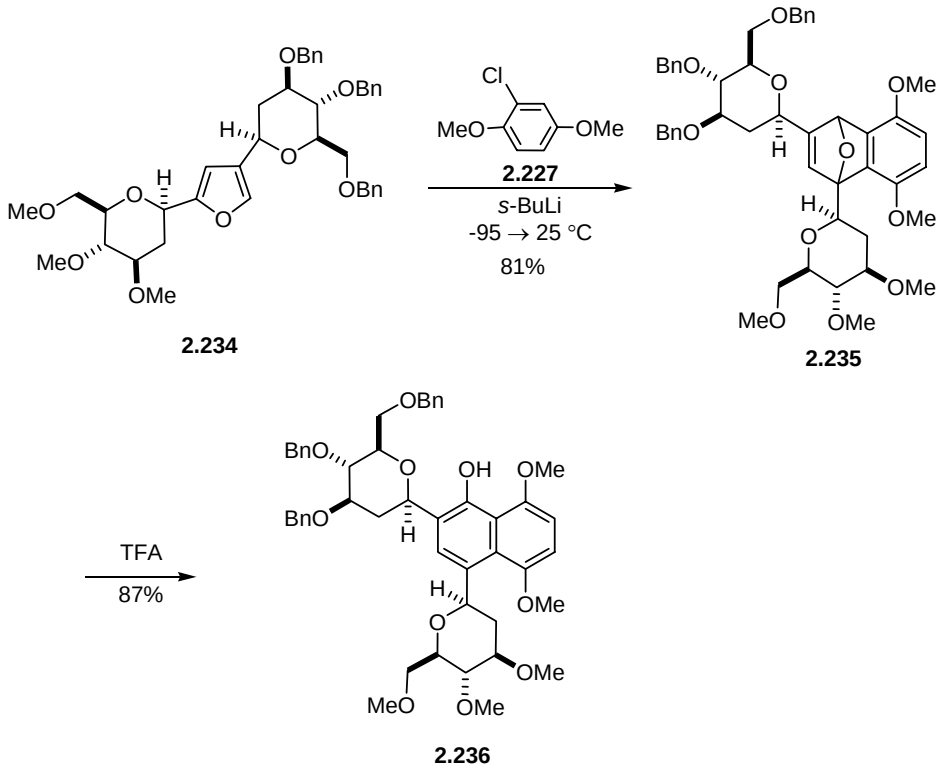
$PhI(OAc)_2$ and subsequent reduction of the resultant quinone with $Na_2S_2O_4$ gave the Group IV *C*-aryl glycoside **2.233** (Scheme 2.56).

Scheme 2.56



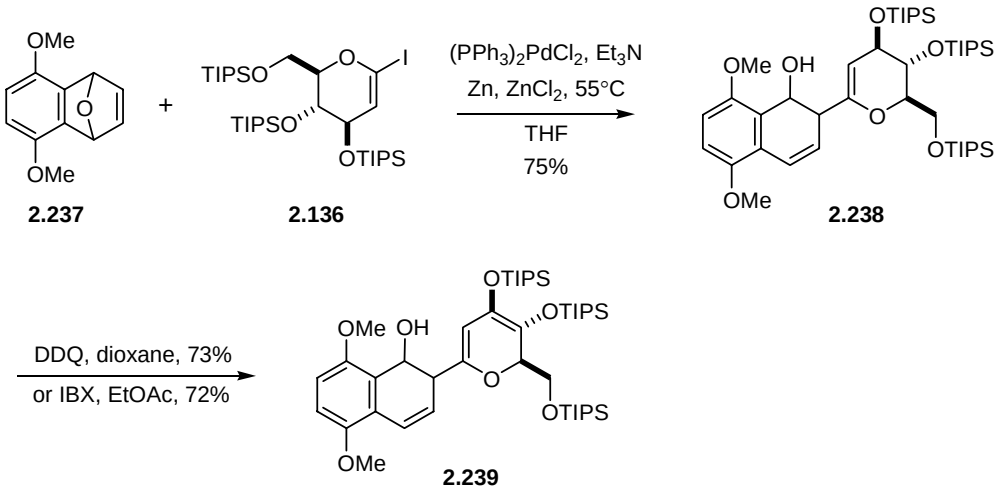
In a similar manner, reaction of 1,4-dimethoxybenzyne with the 1,3-diglycosyl furan **2.234** delivered **2.235,** which was converted to Group III model **2.236** as a single diastereomer upon exposure to trifluoroacetic acid (Scheme 2.57).

Scheme 2.57

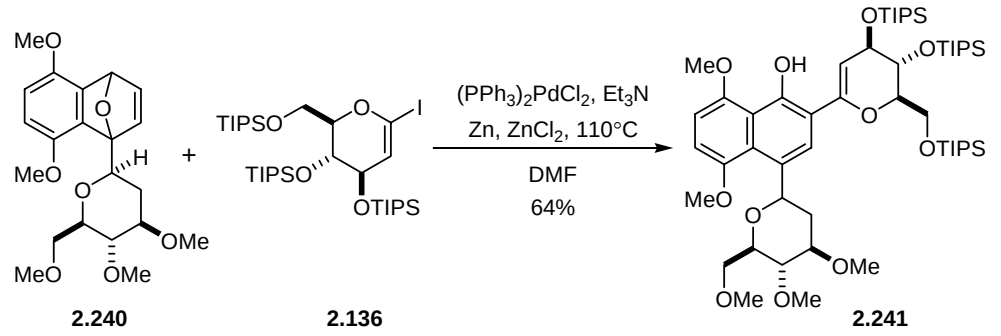


Alternatively, Group I and III *C*-aryl glycosides can also be accessed from S_N2' type ring opening with a sugar nucleophile followed by oxidative aromatization. The palladium-catalyzed reaction of the sugar-substituted cycloadduct **2.237** with **2.136** gave the naphthol **2.238**, which could be oxidized using freshly recrystallized 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ)[269] or IBX[270] to afford Group I model **2.239** (Scheme 2.58). When the cycloadduct **2.240** was subjected to palladium catalyzed S_N2' type opening, Group III model **2.241** was obtained directly without a separate oxidation step (Scheme 2.59).

Scheme 2.58



Scheme 2.59



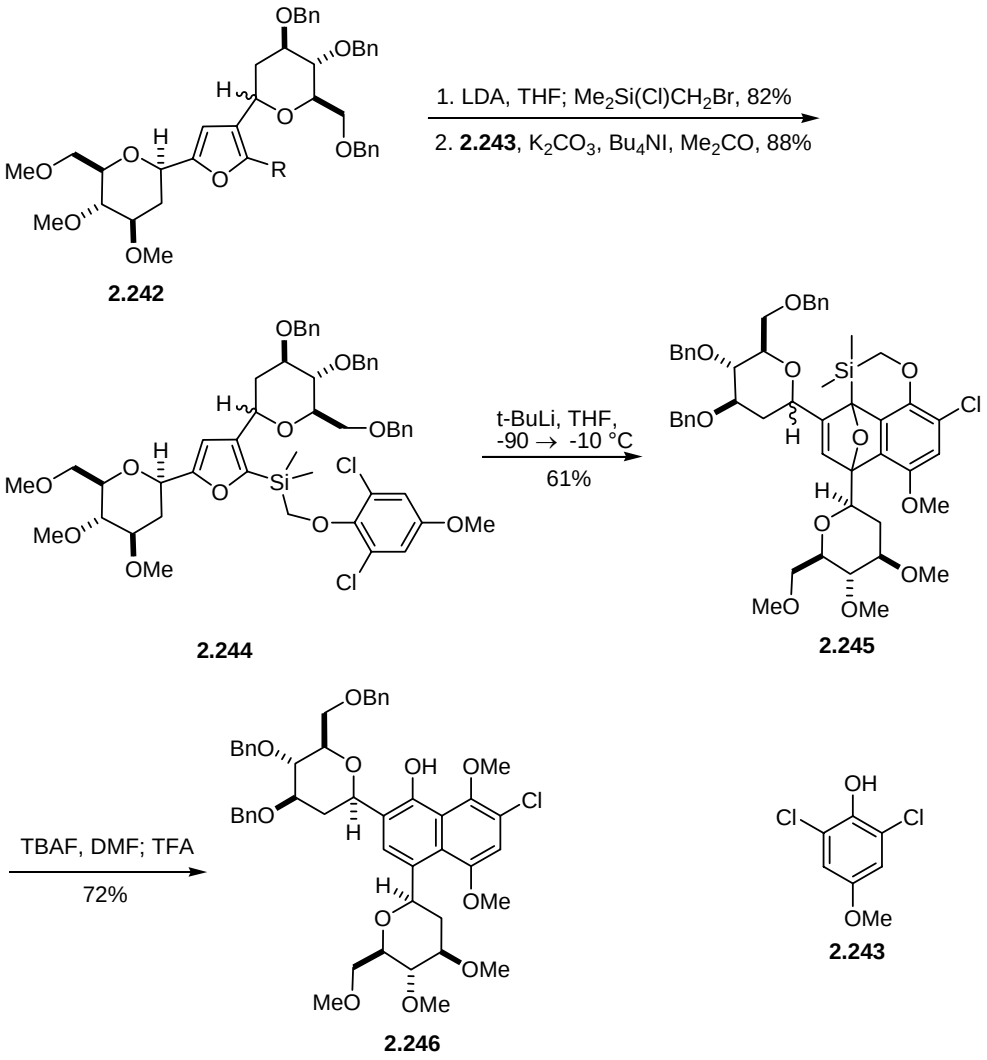
At this stage, the viability of our general entries to the four major groups of *C*-aryl glycoside had been clearly established. All four major *C*-aryl glycosides could be accessed through cycloaddition of benzyne with a properly substituted glycosyl furan following by acid-catalyzed rearrangement of the resultant cycloadducts. Alternatively, Group I and III *C*-aryl glycosides can also be accessed from S_N2' type ring opening with a sugar nucleophile.

2.3.2 The Control of the Regiochemistry Using A Disposable Tether

In the process of establishing the underlying feasibility of the approach, symmetrical benzynes were universally employed as reaction partners, so there was never a regiochemical issue. The issue of global regioselectivity however cannot be ignored shall we apply the method to the syntheses of *C*-aryl glycoside natural products. It should be noted that all of the naturally occurring C-aryl glycoside antibiotics are unsymmetrically substituted, and hence it is essential to control the regiochemistry in the key benzyne-furan cycloaddition.

Literature precedence and research in the Martin group had shown that benzyne-furan cycloadditions was insensitive to steric factors;[271, 272] a disposable silicon tether was therefore used instead. Protocols using silicon tethers with one or two carbon atoms were developed and applied successfully in controlling the regiochemistry of benzyne-furan cycloadditions by former group members Dr. Kaelin and Dr. Sparks and the technique is illustrated in Scheme 2.66.[268]. Thus, α-lithiation of the epimeric 1,3-diglycosyl furans **2.242** followed by silylation provided a furylsilane intermediate, that underwent nucleophilic substitution with phenol **2.243** to produce **2.244**. Selective deprotonation of **2.244** followed by intramolecular benzyne/furan cycloaddition and exhaustive cleavage of the carbon-silicon bonds and acid-catalyzed ring opening delivered the naphthol **2.246** as a single diastereomer (Scheme 2.60).

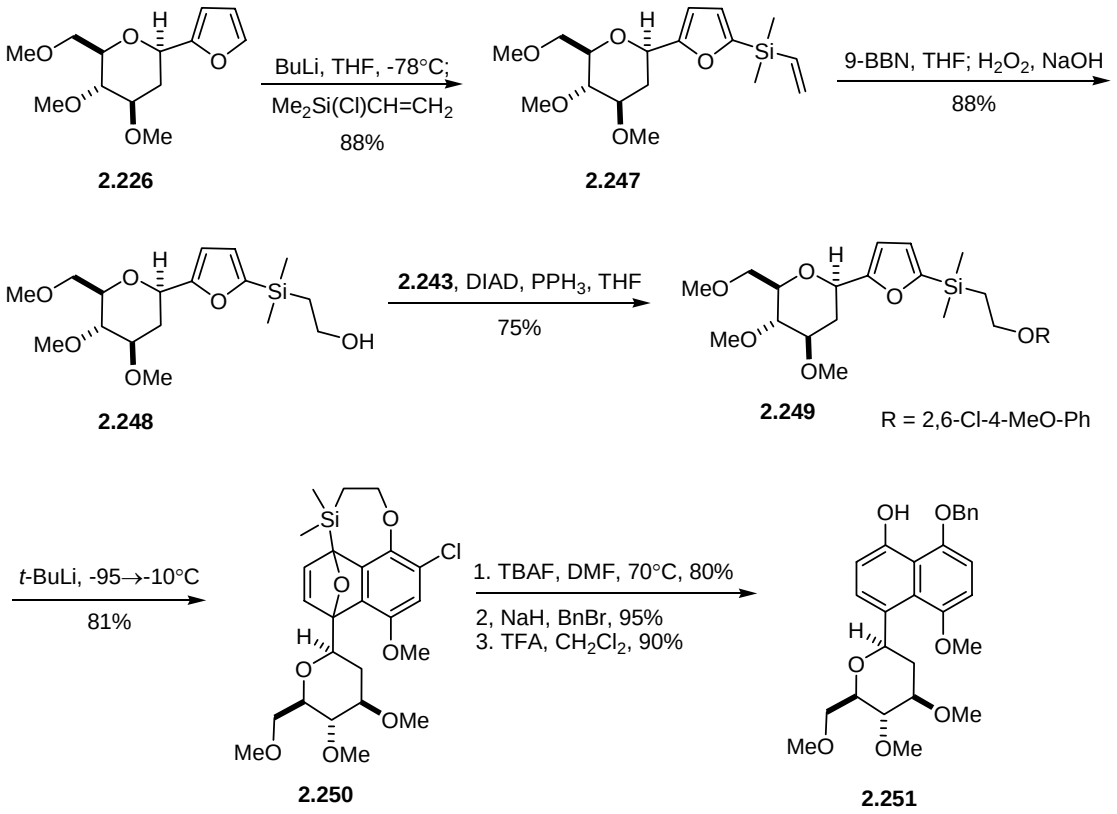
Scheme 2.60



In a similar manner, α-lithiation of the 1-glycosyl furans **2.226** followed by silylation with chlorodimethylvinylsilane provided a furylvinylsilane intermediate **2.247**, which underwent hydroboration/oxidation followed by Mitsunobu etherification with phenol **2.243** to produce **2.249** with a two-carbon tether. Selective deprotonation of **2.249** followed by intramolecular benzyne/furan cycloaddition delivered the cycloadduct **2.250** Cleavage of the silyl tether followed by *O*-alkylation of the resultant alcohol and acid-

catalyzed ring opening gave **2.251** as a single diastereomer in which each of phenolic oxygens is differentiated (Scheme 2.61).

Scheme 2.61



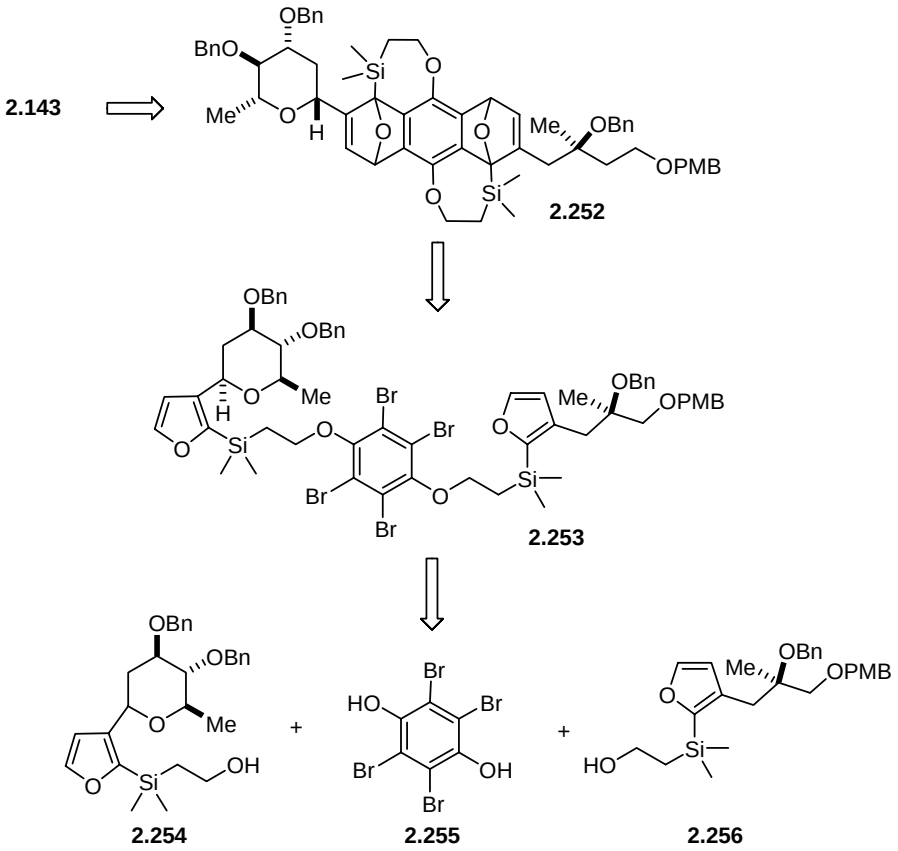
After establishing the regiocontrol by use of a disposable silyl tether in the cycloaddition, efforts in the Martin group shifted towards applying the methodology to the syntheses of naturally occurring *C*-aryl glycosides. In the upcoming section, a brief examination of the synthetic efforts in the Martin group toward vineomycinone B2 methyl ester will be presented.

2.3.3 Synthetic Efforts Toward Vineomycinone B2 Methyl Ester

The vineomycins were isolated from the culture broth of *Streptomyces matensis vineus* and found to be active against Gram-positive bacteria and sarcoma-180 solid tumors in mice.[273, 274] The key structural features of vineomycinone B2 methyl ester are the presence of the β-D-olivose and the 3-(*R*)-hydroxyisovaleryl on opposing ends of an anthrarufin core. A number of total syntheses of vineomycinone B2 methyl ester have been published since its isolation.[227, 275-277]

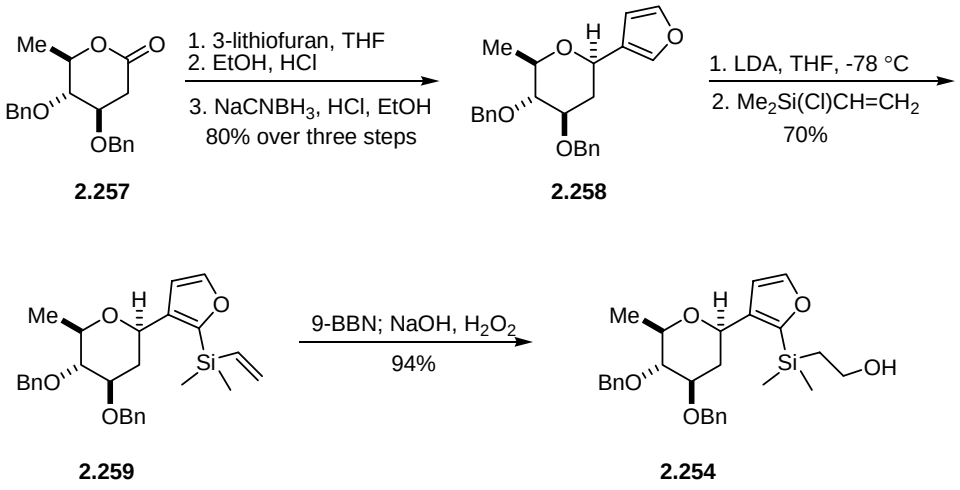
The synthesis of vineomycinone B2 (**2.143**) undertaken by Dr. Sparks relied on a double intramolecular benzyne-furan cycloaddition to form a bisoxabenzonorborna-diene ring system **2.252** in a regio-controlled fashion. Vineomycinone is envisioned to arise from **2.252** *via* tether removal, regioselective ring opening and functional group elaboration (Scheme 2.62).[278]

Scheme 2.62



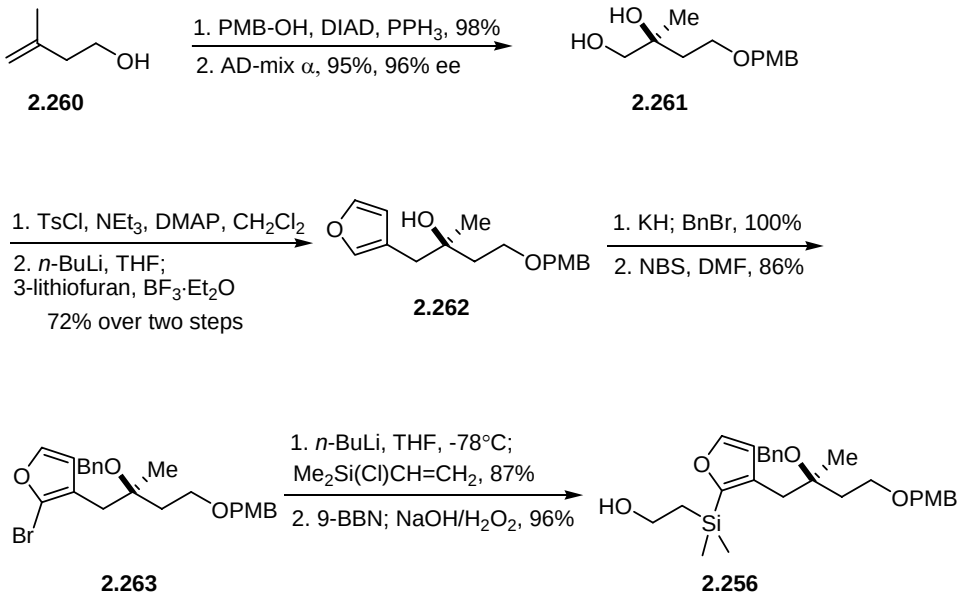
The west potion of the molecule was prepared from the known sugar lactone **2.257**. Addition of 3-lithiofuran to lactone **2.257** followed by stereoselective reduction of the intermediate hemiacetal with $NaCNBH_3$ afforded glycosyl furan **2.258**. α-Lithiation of **2.258** followed by trapping the lithiofuran with chlorodimethylvinylsilane provided vinyl silane **2.259**, which was converted to β-D-olivosyl furan **2.254** by hydroboration/oxidation (Scheme 2.63).[278]

Scheme 2.63



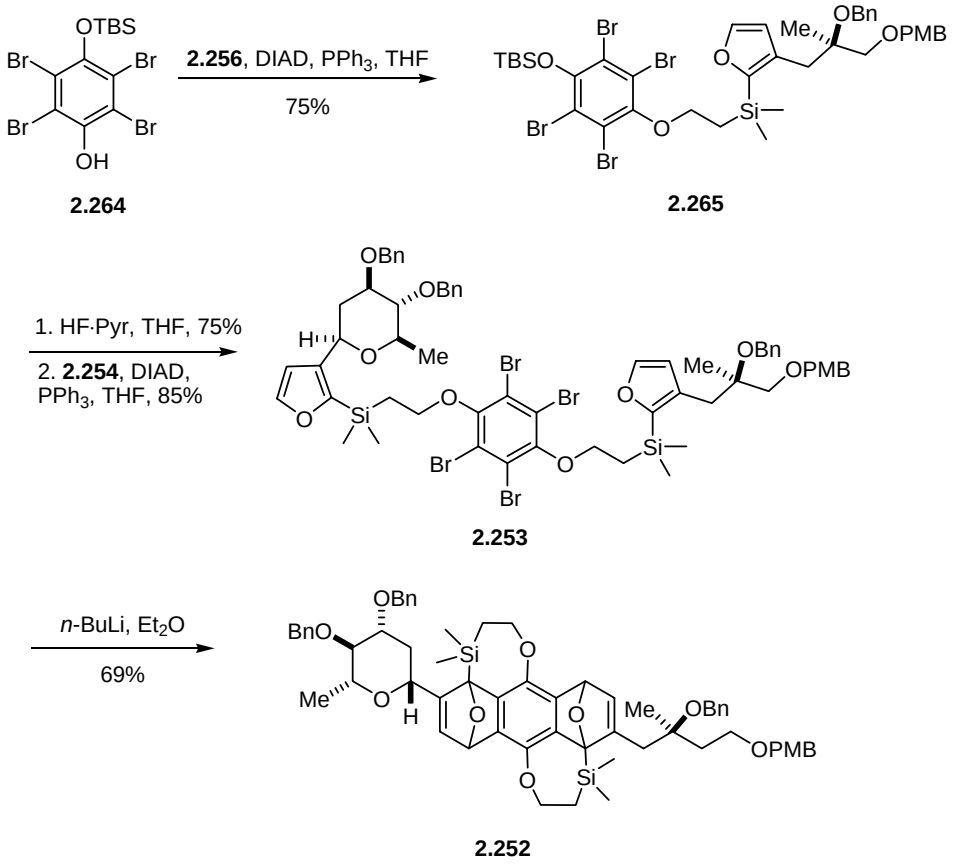
The east potion of vineomycinone B2 was prepared from 3-methyl-3-butenol **2.260** (Scheme 2.64). Protection of **2.260** as its PMB ether followed by Sharpless asymmetric dihydroxylation afforded diol **2.261** in excellent yield and high enantiomeric excess. Diol **2.261** was converted to an epoxide, which was opened with 3-lithiofuran in the presence of BF_3•OEt_2 to provide **2.262**. Benzyl protection of the tertiary alcohol of **2.262** followed by bromination furnished 2-bromofuran **2.263**, which was subjected to metal-halogen exchange, and the resulting anion was trapped with chlorodimethylvinylsilane to afford an intermediate vinylsilane. Hydroboration/oxidation then provided the requisite furan **2.256** (Scheme 2.64).[278]

Scheme 2.64



Mitsunobu etherification of mono-protected tetrabromodihydroquinone **2.264** with alcohol **2.256** provided aryl ether **2.265** in 75% yield. Removal of the silyl protecting group with HF•pyridine followed by a second Mitsunobu etherification delivered **2.253** in excellent yield. Dropwise addition of a dilute solution of *n*-BuLi to a cooled solution of tetrabromide **2.253** in Et_2O effected the crucial double benzyne-furan cycloaddition affording bisoxabenzonorbornadiene **2.252** as a mixture of 4 diastereomers in 68% yield (Scheme 2.65).[278] The final stage of the synthesis entails ring opening and oxidation of the bisbenzonorbornene core followed by functional group manipulation of the side chains to provide vineomycinone B methyl ester.

Scheme 2.65



2.4 CONCLUSION

A brief discussion of the synthetic methods currently available was presented in this chapter. It should be clear at this point the key issues in forming the *C*-aryl glycosyl bond are the control of the regiochemistry and the control of stereochemistry at the anomeric center. The presence of carbohydrate moiety introduces extra chiral centers, multiple Lewis basic sites and steric bulk, thereby complicating this issue of selectivity both sterically and electronically. Furthermore, the ready epimerization of the anomeric carbon in a large number of *C*-aryl glycosides have provided challenges and opportunities

to their syntheses. A number of methods have been developed to address those issues, and majority of them take advantage of the inherent electrophilicity of the anomeric carbon. Methods that provide either anomer reliably and predictably have been reported from a few research groups and a number of naturally occurring *C*-aryl glycosides have been synthesized. Parker, Suzuki and Martin have individually developed systematic approaches to major classes of *C*-aryl glycosides. The upcoming chapter will discuss our efforts in the application of the tactics to the synthesis of *C*-aryl glycoside natural product 5-hydroxyaloin and the further exploration of the benzyne-furan cycloaddition mythology.

Chapter 3: Synthesis and Kinetic Evaluation of Substrate-based Phospholipid Analogues

The major focus of the phospholipase C project within the Martin group has been on studying the mechanistic aspects of the PLC_{Bc}-catalyzed hydrolysis of phospholipids. Prior studies were carried out with the preferred substrate-phosphatidylcholine almost exclusively.[103, 105, 279, 280] The enzyme, however, also hydrolyzes the other two natural substrates-phosphatidylethanolamine and phosphatidylserine with only slightly less efficiency. The ratio of k_{cat}/K_M for enzymatic hydrolysis of the synthetic phospholipids 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine (**1.55a**, C6PC), 1,2-dihexanoyl-*sn*-glycero-3-phospho-ethanolamine (**1.55b**, C6PE), and 1,2-dihexanoyl-*sn*-glycero-3-phospho-L-serine (**1.55c**, C6PS) is 9:6:1.[76] Since the three PLC_{Bc} natural substrates differ only at the polar head groups, the structural differences between the head groups are in one way or another responsible for the differences in specificity. Each substrate bears a positively charged ammonium moiety at physiological pH as part of the head group, while PS also carries a negatively charged carboxylate. With respect to size, the head groups of PC and PS are relatively similar to each other, while the primary ammonium group in PE is significantly smaller. Probably more significantly, PC lacks the capability to act as either a hydrogen bond donor or acceptor. Because it is not clear how PLC_{Bc} recognizes the difference in the head groups among the three very similar phospholipid substrates, efforts were initiated towards developing a better understanding of the structural basis for the substrate specificity of PLC_{Bc}.

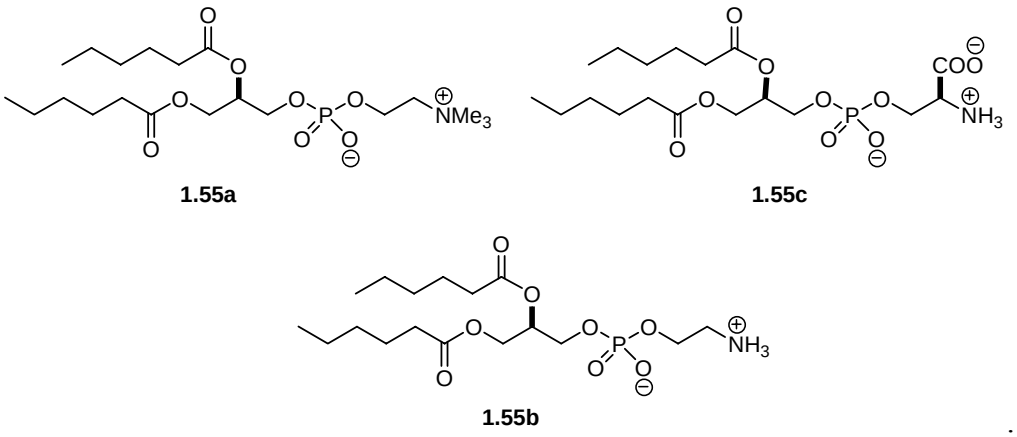


Figure 3.1: Hydrolyzable phospholipids

Initial work in our group by Dr. Antikainen[281] revealed that replacing one or more of the three choline-binding-pocket amino acids residues Glu4, Tyr56, and Phe66 led to the modulation of substrate specificity of PLC_{Bc}.[282] A set of mutants was thus identified that can be used as a starting point to study structure-function relationships. In order to gain a better understanding of the structural factors that are involved in substrate recognization by PLC_{Bc}, it is necessary to examine the three dimensional structure of the wild type enzyme and selected mutants thereof and their complexes with appropriate non-hydrolyzable substrate analogues using X-ray crystallography. It would also be important to obtain the thermodynamic profile of the binding of the enzyme and mutants with non-hydrolyzable substrates using isothermal titration calorimetry (ITC), to semi-quantify the contributions of individual amino acid residues. Structural and thermodynamic studies of such mutant enzymes and their complexes with substrate analogues can lead to a better understanding of the structural basis responsible for the substrate selectivity of PLC_{Bc} towards PC, PE, and PS and may provide valuable insights into the interactions between amino acid side chains in the head group binding pocket of PLC_{Bc} and its substrates.

In order to determine the thermodynamic parameters (ΔG, ΔS, and ΔH) in a binding event accurately by ITC, a specific combination of ligand affinity and concentration are required. For example, a compound having a K_i of 1 mM must be soluble at a concentration of about 2 M.[283] The requirements for X-ray crystallography are less clear, and structures of wild type PLC_{Bc} and its Asp55Asn mutant complexed with a phosphonate inhibitor having a K_i of 1.15 mM and a solubility of 0.5 M in 45% aqueous $(NH_4)_2SO_4$ have been obtained. Therefore, it seems that a phospholipid analogue having solubility of at least 1 mM and K_i below 0.1 mM would be suitable candidate for both techniques.

3.1 Design of Water-Soluble Phospholipids

Prior studies had revealed that replacing one or more of the phosphate oxygen atoms with sulfur led to some of the most potent inhibitors of PLC_{Bc}. For example, the dithiophosphatidylcholine derivative **1.38g** exhibited a K_i of 30 μM[284] or 10 μM[87]. However, the solubility of **1.38g** in water is only 0.2 mM,[285] which is too low to be of practical use in ITC and crystallographic studies. Inhibitors that are more water-soluble were required for further studies. The dithiophosphonate **1.38g** was chosen as the starting point due to the short synthetic route to prepare this type of compounds compared to other thioanaologs, such as **1.38b** and **1.38f**.

Preparing dithiophosphatidylcholine analogues of **1.38g** having greater water solubility would be only a matter of either increasing the overall hydrophilicity by installing of polar functionalities or decreasing the overall lipophilicity of the molecule by truncation of non-polar functionalities. The three-dimensional crystal structure of PC-PLC from *B. cereus*[80] and its D55N mutant[106] complexed with nonhydrolyzable substrate analogues revealed extensive interactions between the amino acid residues and

the phosphate and choline head group moieties of the substrate analog. Modification of this potion of the molecule would thus be expected to deprive the substrate analog of the ability to bind to PLC_{Bc}. The crystal structure also revealed the lack of any obvious interactions between two acyl side chains and the enzyme except for the presence of a distinct hydrogen bonding between the *sn*2-carbonyl and asparagine 134. Both fatty acid tails were somewhat disordered with thermal motion increasing along the aliphatic side chain. The termini of the lipophilic side chains appeared to be completely solvent accessible. Thus it seemed reasonable that modifications to the termini of the *sn*-1 and *sn*-2 acyl side chain would have a minimal impact on the binding affinity of the substrate analog. On the other hand, kinetic experiments have shown that shortening the lipophilic side chains is detrimental to K_M of phospholipid substrate. Therefore, analogues with shorter side chains would likely be less potent inhibitors.

There are two possible approaches to maintain longer side chain(s) while increasing water solubility. The first of these involves partial or complete removal of the lipophilic *sn*-1 side chain, which was not involved in any identified significant interactions in the crystal structure.[80] In fact, phospholipid derivatives bearing a single acyl chain have been shown to be substrates for PLC_{Bc}.[286] Therefore, it is reasonable to believe a non-hydrolyzable substrate analog with only *sn*-2 side chain, such as **3.4** could be a potential inhibitor that is more soluble than its diacyl counterpart. The second tactic would involve installing highly hydrophilic substituent(s), such as hydroxyl, amino and carboxyl groups, at the end of one or both of the acyl side chains to increase water solubility. Because termini of the side chains are exposed to solvent in the lipid-enzyme complex, the presence of terminal polar groups was not expected to adversely affect binding. Hence, dithiophosphatidylcholines possessing hydroxyl groups at the terminus of each acyl side chain were identified as targets. It was expected that screening of

analogs with different side-chain lengths would be needed to produce the best inhibitor due to the correlation between the potency of the inhibitor (K_i), the solubility and the length of the lipophilic side chains.

Inasmuch as one of the long-range goals of this project was to correlate enzyme structure with specificity, the related series of phosphatidylcholine **3.3**, the phosphatidylethanolamine **3.4**, and the phosphatidyl-L-serine **3.5**, were chosen as the initial targets for investigation.

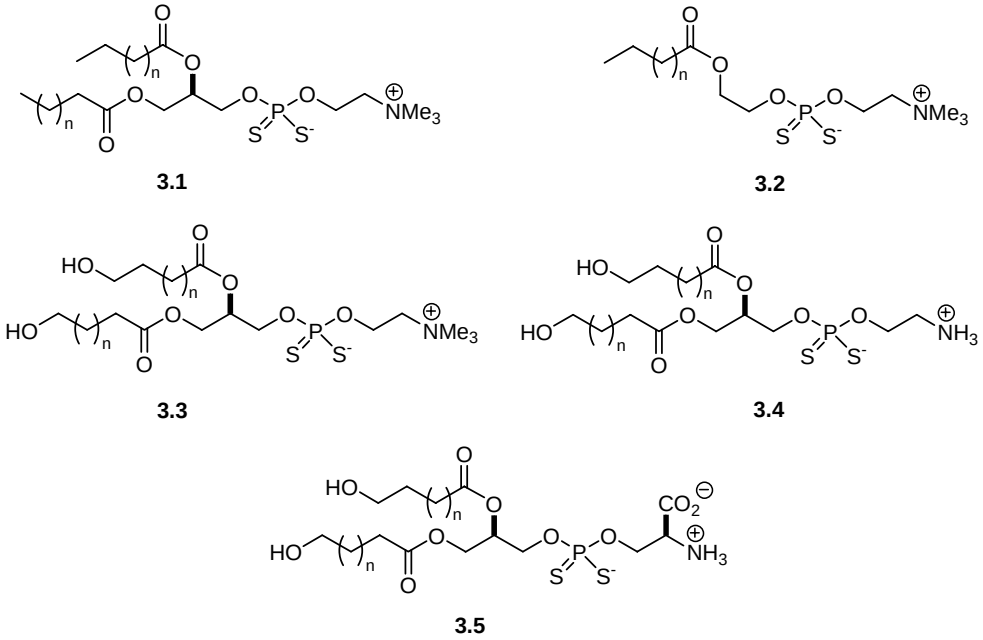


Figure 3.2: Non-hydrolyzable phospholipid analogs

3.2 Synthesis of Water-Soluble Phospholipids

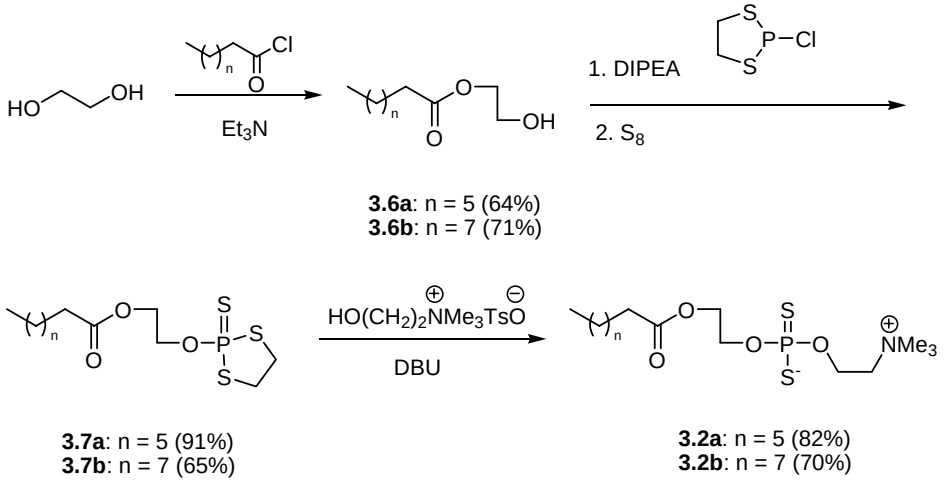
The phosphorodithioate inhibitors **3.3-3.5** that were chosen as the targets were synthesized using a chlorophosphite coupling developed in our lab (Scheme 3.1).[130] This

protocol employs the reactive 2-chloro-1,3,2-dithiaphospholane followed by sulfuration and is extremely efficient for syntheses of various phospholipid analogs.

3.2.1 Ethylene-Glycol Derived Cholinephosphorodithioate

Chris Franklin synthesized the ethylene glycol-derived phospholipid analogues **3.2a** and **3.2b** according to the sequence of reactions depicted in Scheme 3.1.[287] The acid chlorides were treated with an excess of ethylene glycol to give monoesters **3.6a** and **3.6b**, respectively. The alcohols **3.6a** and **3.6b** were then converted into **3.2a** and **3.2b**, respectively, *via* their corresponding thiophosphorodithiolanes **3.7a** and **3.7b** using the oxidative chlorophosphite coupling condition previously established in this group.[130]

Scheme 3.1



The ethylene glycol-derived phosphocholines **3.2a** and **3.2b** were both good inhibitors, exhibiting similar K_i's and solubilities (Table 3.1). Hence, the presence of an *sn*-1 fatty acid subunit is neither mandatory for substrates of PLC_{Bc},[286] nor it is an essential structural requirement for inhibitors.

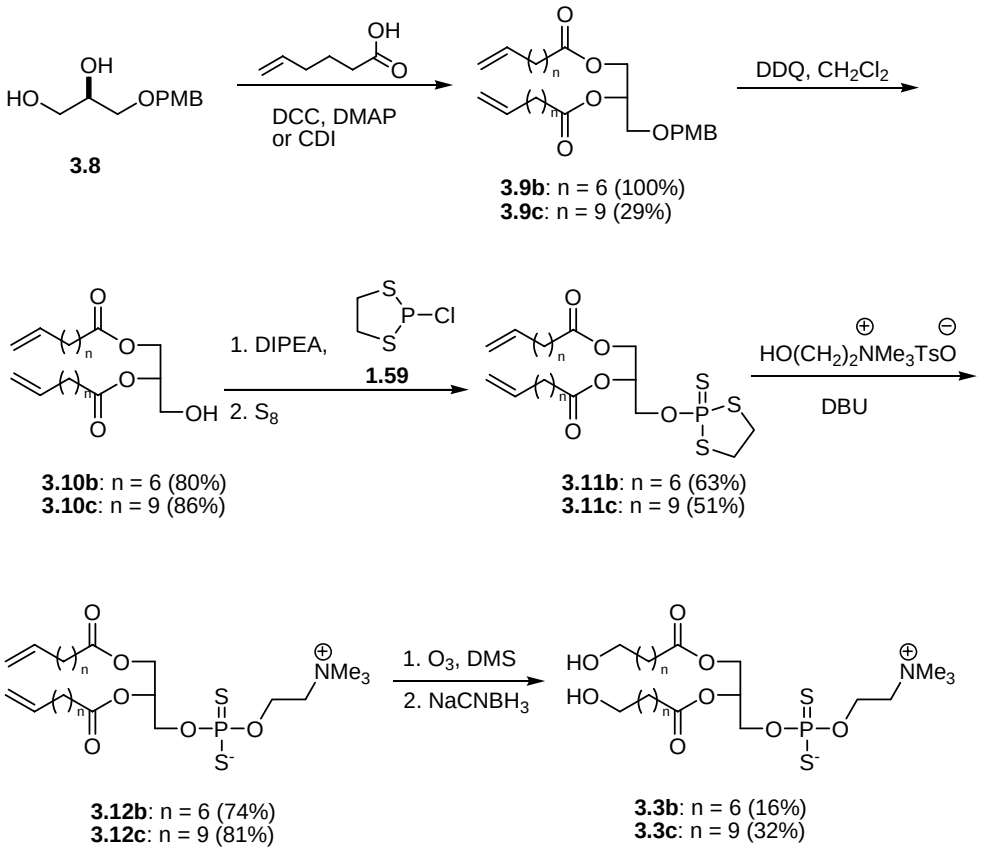
Table 3.1: Results of Kinetic and Solubility Studies on Ethylene Glycol Derived Phospholipid Analogues

Phospholipid	Solubility (mM)	K_i (mM)	Enzyme
3.2a	3	0.51	PLC_{Wt}
3.2b	1	0.42	PLC_{Wt}

3.2.2 ω-Hydroxy Cholinephosphorodithioate

The synthesis of the ω-hydroxy phosphorodithioates **3.3**-**3.5** required the design of a protocol for introducing a hydroxyl group on the terminus of the acyl side chain, Chris Franklin adopted a strategy in which a terminal alkene was used as the latent hydroxyl functional group. (*R*)-3-(4-Methoxybenzyloxy)propane-1,2-diol (**3.8**), prepared according to literature procedure,[112, 117] was diacylated with ω-unsaturated fatty acid, prepared from the corresponding bromide, to afford **3.9b-c** (Scheme 3.2). Oxidative deprotection of the 4-methoxybenzyl (PMB) ether with dichlorodicyano-benzoquinone (DDQ) proceeded smoothly to furnish the alcohols **3.10b-c**, which were immediately used in the next steps to avoid 1,2-acyl migration.[288] The 4-methoxylbenzyl was chosen as the protective group instead of benzyl because it can be removed in the presence of double bonds.[114] Oxidative phosphorylation of **3.10b-c** using 2-chloro-1,3,2-dithiaphospholane **1.59** and sulfur followed by treatment of the intermediates **3.11b-c** with choline tosylate in the presence of DBU provided the unsaturated phosphatidylcholine derivatives **3.12b-c** in good overall yields. Ozonolysis of the terminal double bonds and subsequent reduction of the intermediate aldehyde using sodium cyanoborohydride provided the ω-hydroxy phosphorodithiocholines **3.3b-c** in low yields (Scheme 3.2).

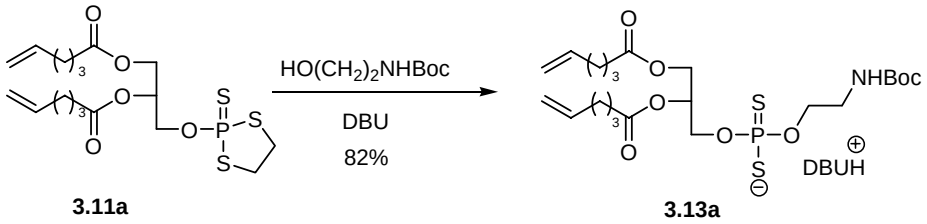
Scheme 3.2



3.2.3 Ethanolamine- and Serine Phosphorodithioates

It was initially expected that the corresponding ethanolamine and L-serine derivatives could be prepared similarly by using a terminal alkene as the latent hydroxyl functional group. Toward this goal, the protected ethanolamine analog **3.13a** was prepared by opening 1,3,2-dithiaphospholane **3.11a** with *N*-Boc ethanolamine (Scheme 3.3).

Scheme 3.3

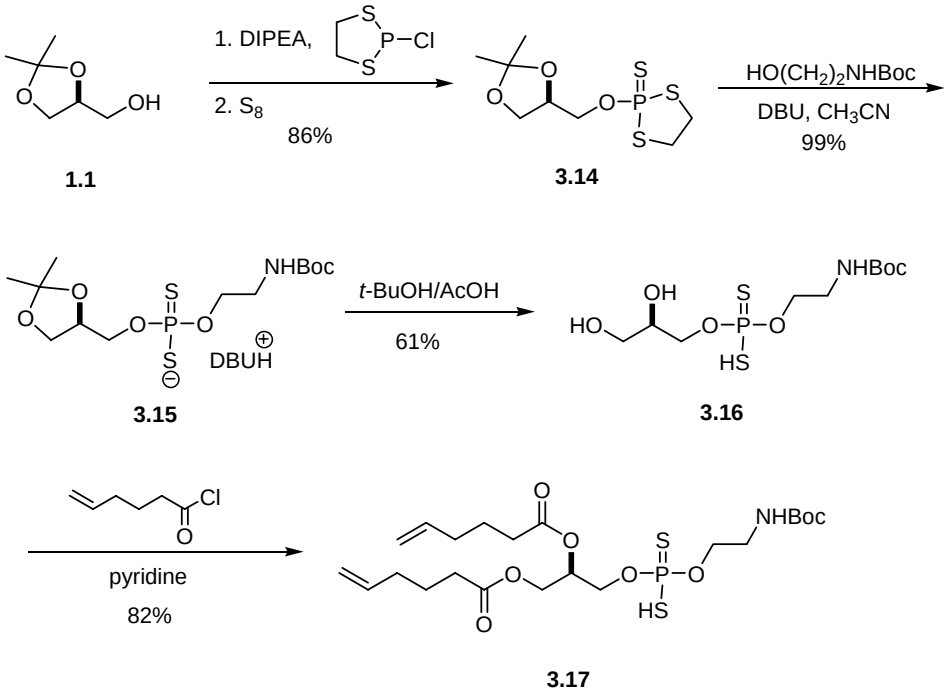


3.11a **3.13a**

The synthetic route to **3.3b-c** enabled us to install different head groups at the end of the synthesis, thereby allowing the preparation of phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidyl L-serine (PS) from a common intermediate **3.11b-c**. However the priority of project was to screen for the best inhibitors, which would have low K_i and high aqueous solubility. Because the length of the fatty acid side chain is the likely determinative factor. The strategy in which the fatty acid side chains were installed in the first step of the syntheses is ill fitted for the purpose of synthesizing a number of analogs with different lengths of side chains. Therefore, a different approach was developed so that different fatty acids could be installed at a later stage of the synthesis.

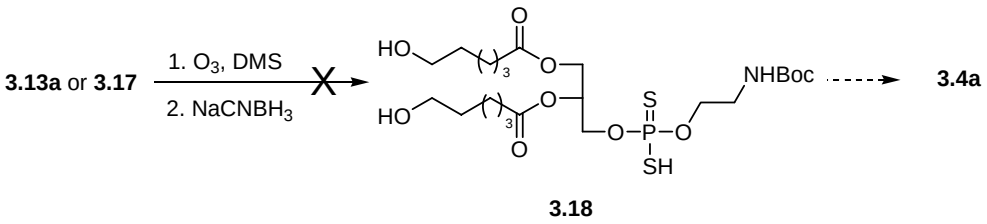
The known 2,3-*O*-isopropylidene-glycerol (**1.1**)[117] was subjected to phosphite coupling conditions to deliver dithiaphospholane **3.14** in good yield. Upon exposure of **3.14** to *N*-Boc-ethanolamine in the presence of DBU, the phosphatidylethanolamine derivative **3.15** was obtained quantitatively. Removal of the acetonide with acetic acid in *t*-BuOH afforded the 1,2-diol **3.16**, which was subsequently diacylated with the appropriate acid chloride in neat pyridine to give the unsaturated phosphatidylethanolamine derivative **3.17** in good yield. This approach had an additional advantage that protection of the C3-hydroxyl was no longer required (Scheme 3.4).

Scheme 3.4



As described previously, ozonolysis of **3.12b-c** followed by reduction provided ω-hydroxyl choline phosphorodithioates **3.3b-c**, albeit in very poor yields. The procedure, however, resulted in concurrent partial or complete replacement of sulfur with oxygen in the dithiophosphate moiety when ethanolmine derivatives **3.17** and its DBU salt **3.13a** were used (Scheme 3.5). The oxidation of sulfur was clearly a competing process. The ^{31}P NMR spectrum revealed three peaks with chemical shifts at 126, 63, 5 ppm in an approximate 2.5:2:4 ratio in a carefully controlled ozonolysis using Sudan III dye as end-point indicator.[289] Assuming the peak at 126 ppm corresponded to the desired product, the isolated yield was at best no more than 10%. In addition, the reaction mixture is difficult to purify, and the fractions after column chromatograph were often contaminated with boron-containing species.

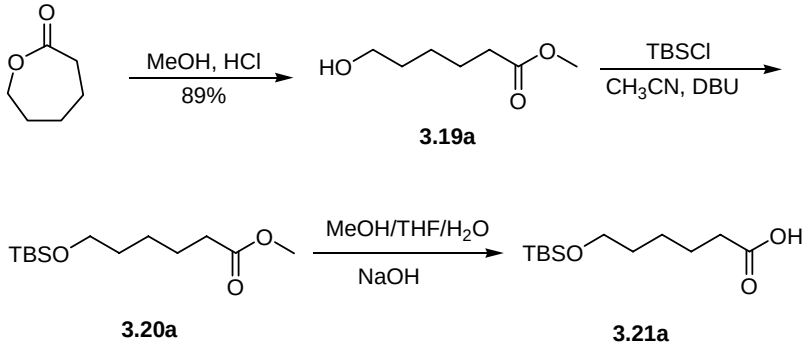
Scheme 3.5



3.18

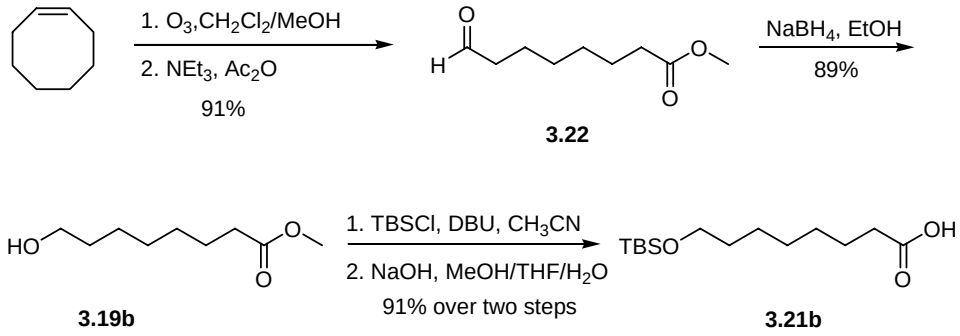
In order to develop a more efficient approach to compounds related to **3.3**-**3.5,** a strategy in which a protected hydroxyl group was present from the onset of the synthetic sequence was examined so a late-stage oxidation could be avoided. The newer approach was expected to be similar in length due to the added protection/deprotection steps. Thus, protected hydroxy acids were used as starting materials. Caprolactone was subjected to acid-catalyzed methanolysis, and the free alcohol of the resulting hydroxy ester **3.19a** was protected as its TBDMS ether.[290] The methyl ester was then saponified with aqueous NaOH to afford the desired *O*-protected ω-hydroxyl carboxylic acid **3.21a** (Scheme 3.6). Alternatively, the terminal differentiated ω-carbonyl carboxylic acid **3.22** was prepared *via* the nonsymmetrical ozonolysis protocol developed by Schreiber.[291] Further functional group manipulation delivered *O*-silyloxy ω-hydroxyl carboxylic acid **3.21b** (Scheme 3.7).

Scheme 3.6



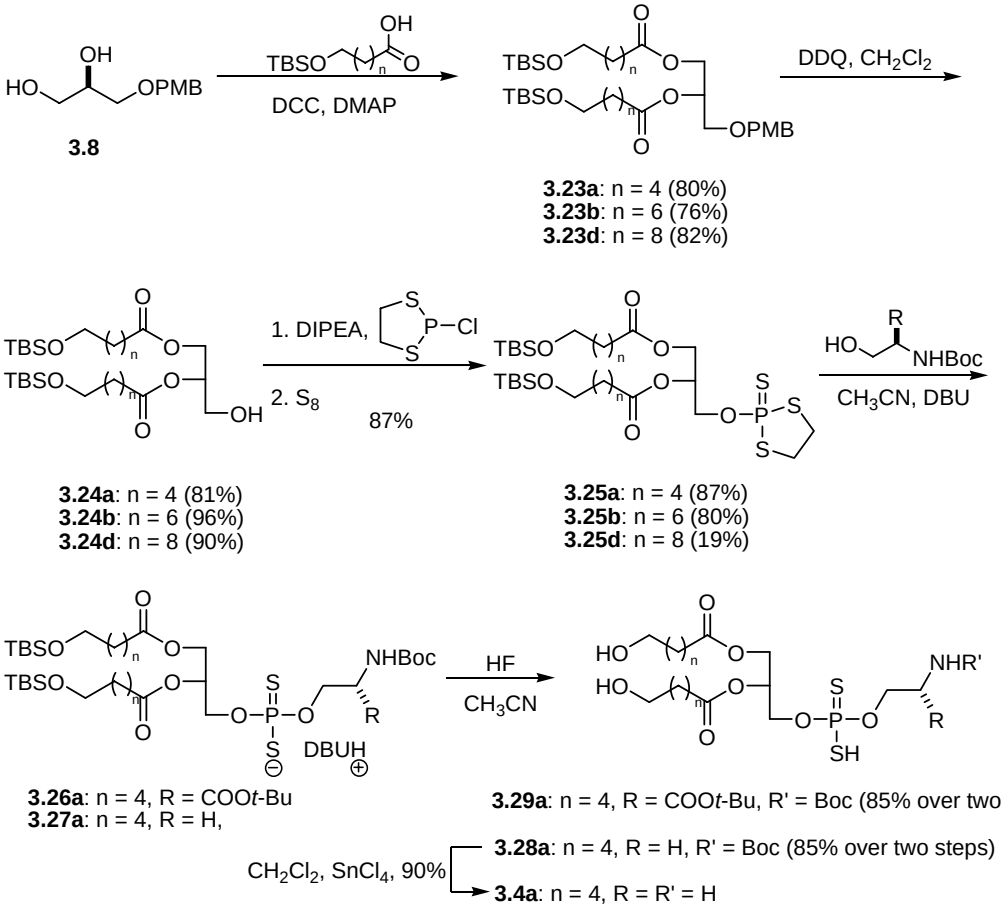
3.19a **3.20a** **3.21a**

Scheme 3.7

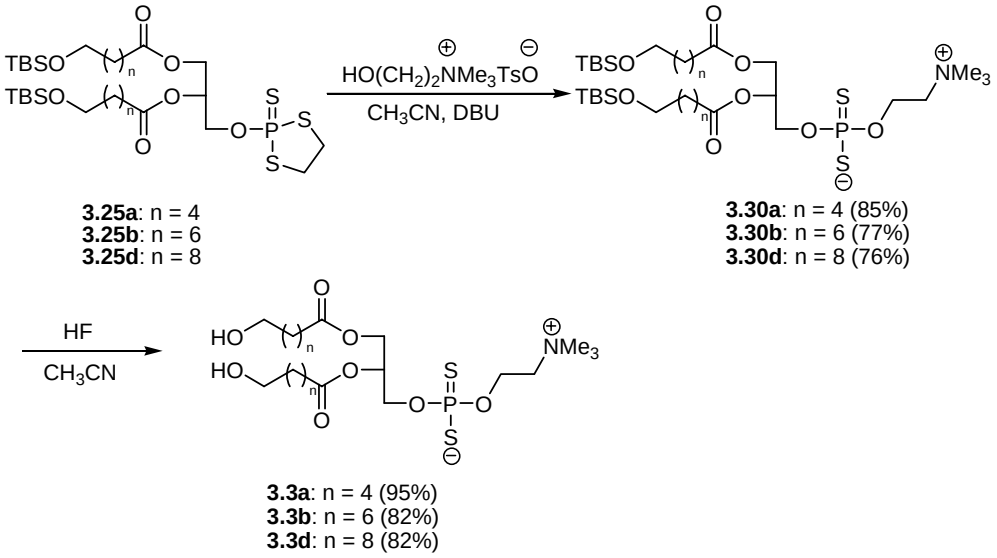


Diacylation of the 1,2-diol **3.8** with protected ω-hydroxyl acid **3.21a** using DCC/DMAP coupling followed by removal of the PMB protecting group with DDQ gave **3.24a**, which was converted into the thiophosphorodithiolane **3.25a** in the usual manner by dithiophosphite coupling and sulfurization. Reaction of **3.25a** with choline tosylate or *N*-Boc ethanolamine in the presence of DBU followed by unmasking of the terminal hydroxyl group using dilute hydrofluoric acid[292] provided the requisite dithiophosphatidylcholine **3.3a** (Scheme 3.9**)** and the partially protected ethanolamine analogues **3.28a** (Scheme 3.8**)**, respectively. *N*-Deprotection of **3.28a** was effected with $SnCl_4$[293] to deliver dithiophosphatidylethanolamine **3.4a** (Scheme 3.14**)**, while global deprotection with TFA/CH_2Cl_2 gave inferior result.

Scheme 3.8


OH
HO
OPMB
3.8
TBSO
OH
n
O
DCC, DMAP
TBSO
TBSO
DDQ, CH2Cl2
OPMB
3.23a: n = 4 (80%)
3.23b: n = 6 (76%)
3.23d: n = 8 (82%)
1. DIPEA,
P−Cl
2. S8
87%
OH
3.24a: n = 4 (81%)
3.24b: n = 6 (96%)
3.24d: n = 8 (90%)
3.25a: n = 4 (87%)
3.25b: n = 6 (80%)
3.25d: n = 8 (19%)
HO
NHBoc
CH3CN, DBU
NHBoc
R
DBUH
3.26a: n = 4, R = COOt-Bu
3.27a: n = 4, R = H,
HF
CH3CN
NHR'
SH
3.29a: n = 4, R = COOt-Bu, R' = Boc (85% over two steps)
CH2Cl2, SnCl4, 90%
3.28a: n = 4, R = H, R' = Boc (85% over two steps)
3.4a: n = 4, R = R' = H

Scheme 3.9

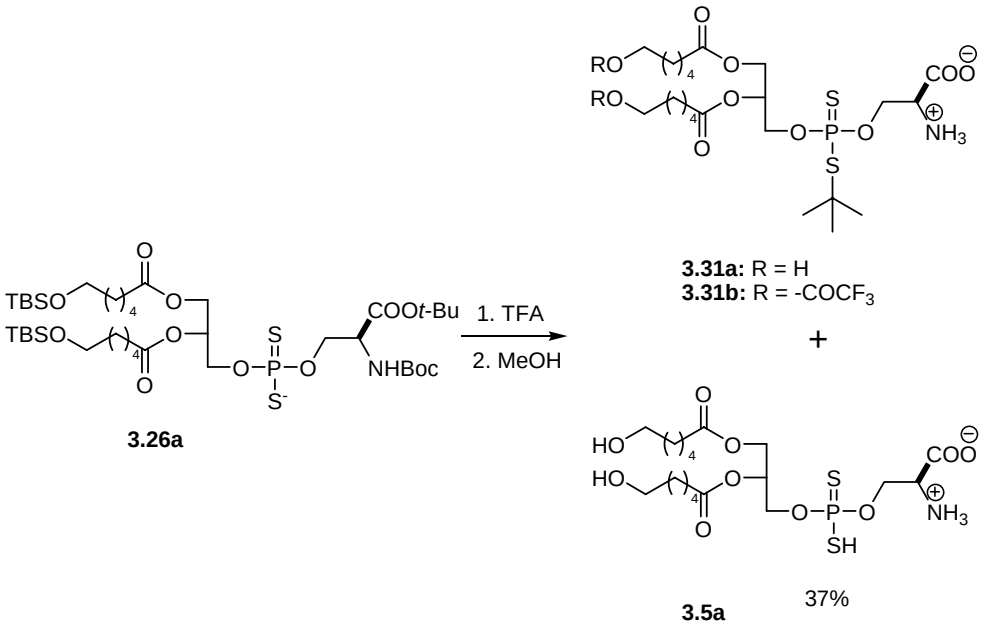


The success in preparing L-serine analog **3.36** lay in the selection of the proper protective group for the carboxyl group. We had originally envisioned that the acid group of serine might be best protected as its *tert*-butyl ester because both the *tert*-butyl ester and the Boc group could be removed in a single operation. However, cleavage of the *tert*-butyl ester from **3.29a** required more forcing conditions than was necessary to remove the *N*-Boc group, and the deprotection of **3.29a** was never clean.

The deprotection of **3.26a** and **3.29a** was complicated by two issues. 1) Both terminal hydroxyl groups were esterified in the presence of excess organic acid (TFA or formic acid), as confirmed by FAB-MS and ^{1}H. The trifluroacetate could be hydrolyzed by simply stirring in methanol (Scheme 3.10). 2) The released *t*-butyl cation was partially trapped by the dithiophosphate ester, even in the presence of large excesses of cation scavengers such as Et_3SiH,[294] anisole, ethanedithiol and/or thioanisole. In the very best case, the contaminant **3.31b** was reduced to ca. 5% (based on ^{1}H NMR) from typical 20% when the solvent was thioanisole and TFA (1:1) and 20 equivalents of ethanedithiol

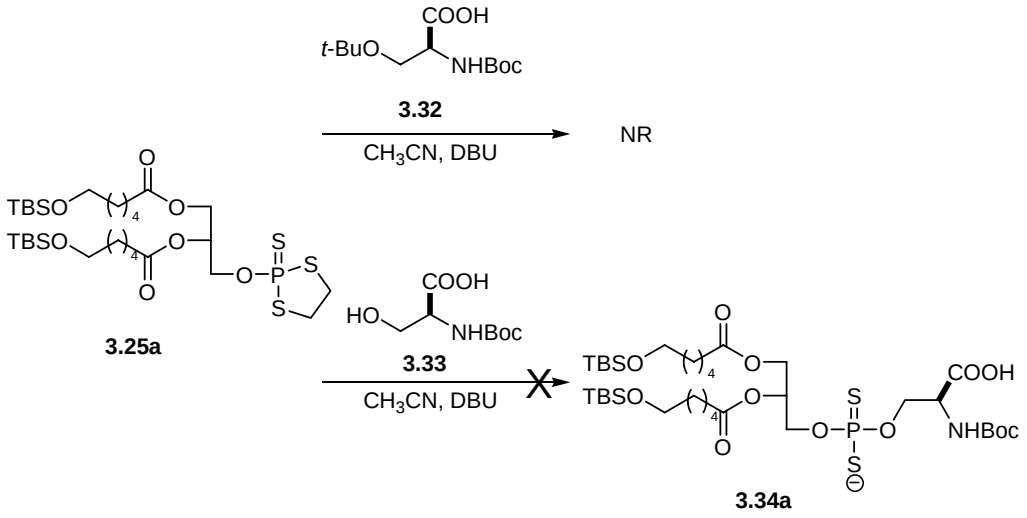
were added. The desired product **3.5a** could be purified using reverse-phase HPLC or tedious flash column chromatography.

Scheme 3.10



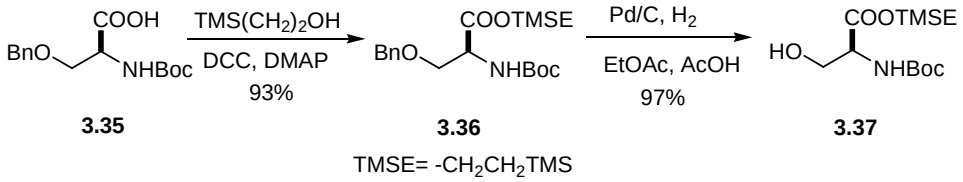
At this point, we wondered whether the protection of the carboxyl group was truly necessary. Namely, could the phosphorylation proceed in the presence of an unprotected carboxylic acid? In a preliminary experiment to address this question, dithiaphospholane **3.25a** was found not to react with *N*-Boc-Ser(O*t*-Bu)-OH (**3.32**) in the presence of DBU (1 equivalent) and both dithiaphospholane **3.25a** and the serine **3.32** were recovered cleanly after the reaction, suggesting that the free carboxyl group alone was not reactive in the phosphorylation. However, a later attempt with derivatized serine **3.33** failed to deliver the desired product **3.34a**. Clearly, the presence of carboxylic acid did interfere with the phosphorylation of the alcohol and it had to be protected (Scheme 3.11).

Scheme 3.11



The search for an easily removable protective group in place of *tert*-butyl ester finally ended up with trimethylsilylethyl ester[295] as the protective group of choice. The syntheses of the requisite alcohol **3.37** followed literature precedents (Scheme 3.12).

Scheme 3.12



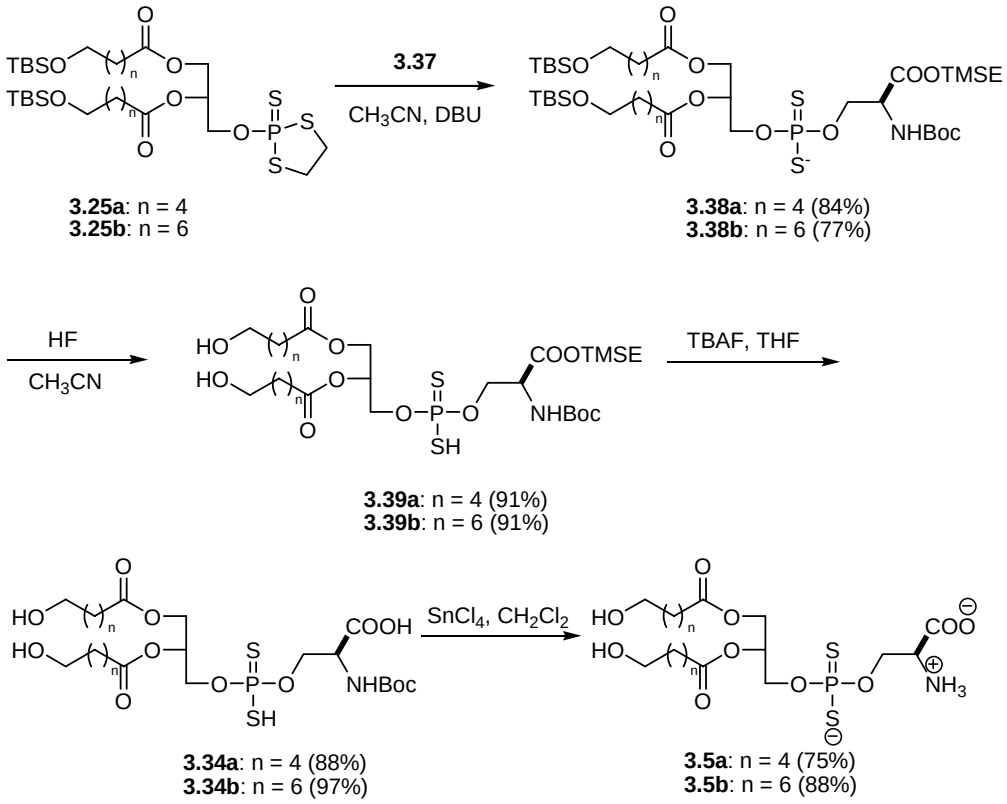
3.35 **3.36** **3.37**

TMSE= -CH_2CH_2TMS

Sequential treatment of **3.38a-b**, which were obtained from **3.25a-b** after chlorophosphite coupling and reaction with **3.37**, with tetrabutylammonium fluoride (TBAF) and $SnCl_4$ cleanly removed carboxyl and amino protective group to deliver the desired L-serine phosphorodithioate **3.5a** and its C8 analog **3.5b** (Scheme 3.13). Though it was possible to remove the terminal silyl ether and trimethylsilylethyl ester from **3.38a-**

b concurrently with TBAF, the yield was lower than the two-step procedure and chromatography purification was difficult.

Scheme 3.13

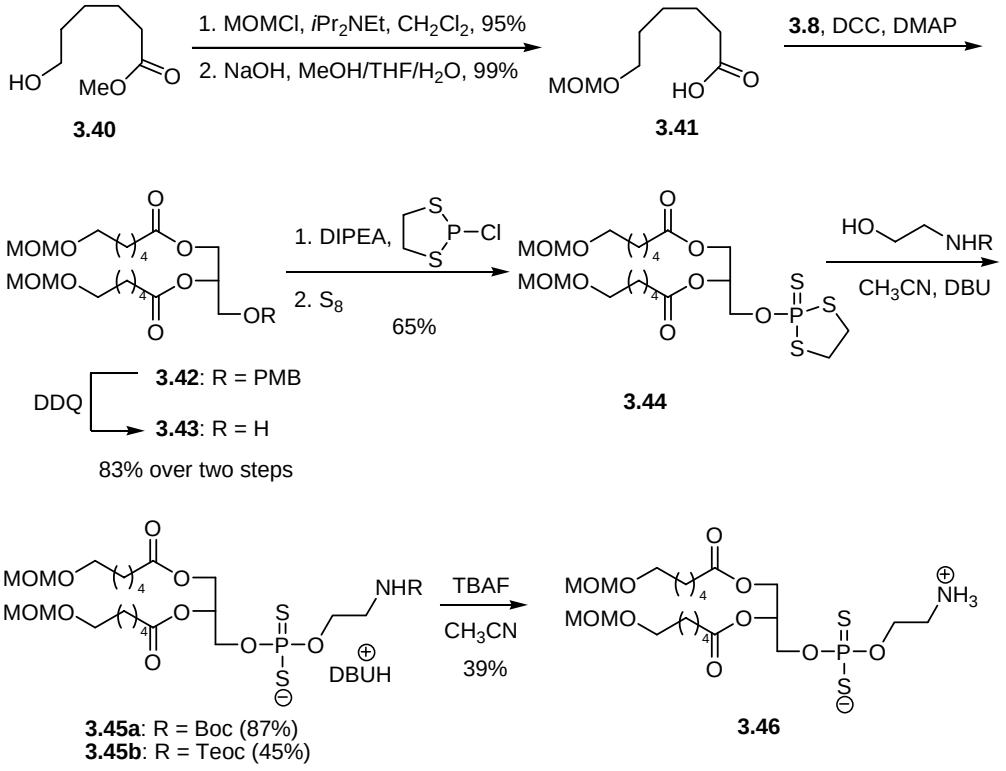


3.2.4 Other Phosphorodithioate

Meanwhile, it was thought a polar functionality isosteric to an alkyl group could mimic the fatty acid tails to improve water solubility without impairing the inhibition potency. Thus the synthesis of **3.46** was undertaken. Protected ω-methoxymethoxy ethanolamine phosphorodithioate **3.45b** was prepared from **3.8** in the usual manner (Scheme 3.14). Removal of the trimethylsilylethyl carbamate protective group (Teoc) of **3.45b** was achieved with TBAF in CH_3CN affording dithiophosphatidylethanolamine

3.46. The *tert*-butyl carbamate analog **3.45a** could not be converted into **3.46** because deprotection of *N*-Boc was always accompanied with concurrent loss of the terminal methoxymethyl ethers.

Scheme 3.14



3.2.5 Phosphonate

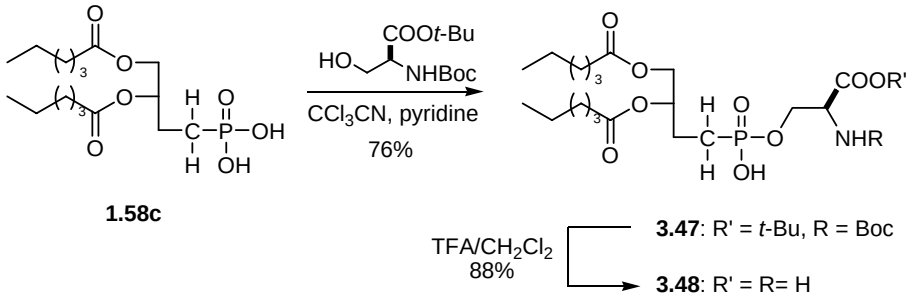
Nina Antikainen obtained complexes of D55N mutant of PLC_{Bc} with C6-cholinephosphonate **1.38c** and C5-choline phosphorodithioate **1.38h**. The two analogs are nearly equipotent as inhibitors with inhibition constant (K_i) being 1.15 M for **1.38c**[76] and 1.21 M for **1.38h**[287]. Similar level of active site occupancy (~80%) in the crystal structure of D55N was observed even though phosphorodithioate **1.38h** was about 10 times more soluble than choline phosphonate **1.38c** (5 mM versus 0.5 mM) under the

soaking conditions.[106] It was suggested that the phosphorodithioate might not be the best candidate for X-ray crystallization studies and phosphonate might be better suited for this purpose. Therefore the syntheses of phosphonate serine **3.48** and ethanolamine analogs **3.53** were undertaken.

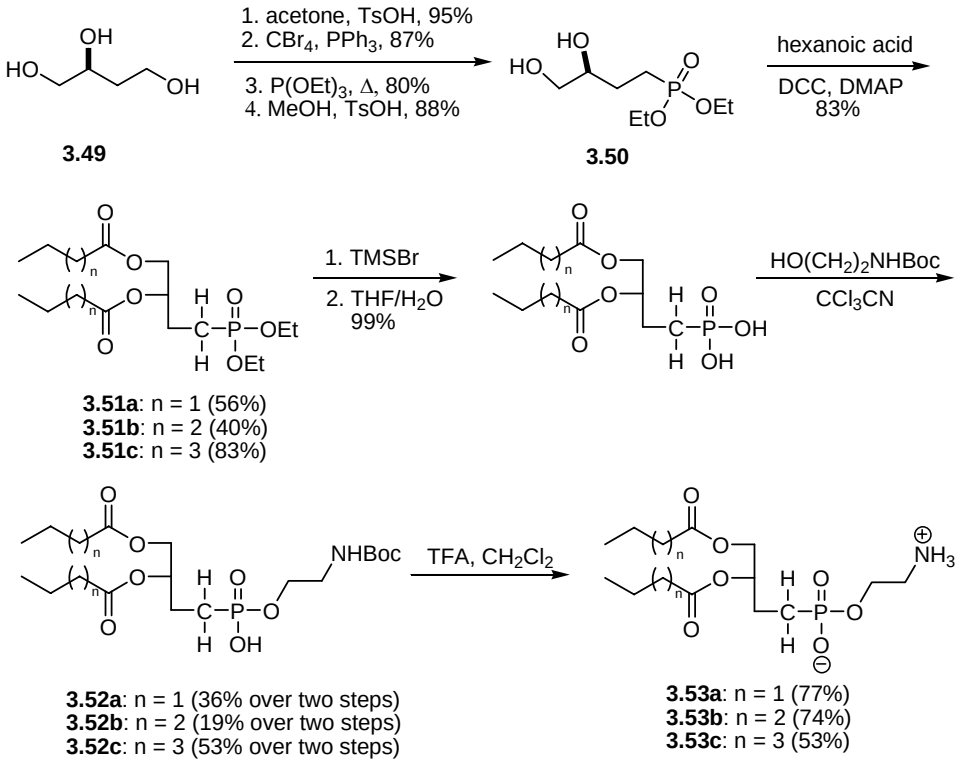
The synthesis of L-serine phosphonate **3.48** closely followed that of **1.38c** published by Martin and Wong.[87] Thus, phosphonic acid **1.58c** and *N*-Boc-ser-O*t*-Bu were coupled in the presence of CCl_3CN or 2,4,6-trisopropylbenzenesulfonylchloride followed by global deprotection of the carbamate and *tert*-butyl ester with TFA in CH_2Cl_2 to afford the desired phosphonate **3.48** in 67% yield over two steps (Scheme 3.15).

The phosphonate ethanolamine **3.53c** was prepared similarly, from phosphonic acid **3.29c** and *N*-Boc-ethanolamine followed by deprotection of the intermediate carbamate **3.52c** with TFA in CH_2Cl_2. It was later found that **3.53c** was too insoluble, so analogs **3.52a** and **3.52b** with shorter-carbon chains (C4 and C5) were prepared using identical chemistry (Scheme 3.16).

Scheme 3.15



Scheme 3.16



3.3 KINETIC EVALUATION OF WATER-SOLUBLE PHOSPHOLIPIDS

With several substrate-based analogues in hand, the immediate task was to evaluate them as inhibitors of PLC_{Bc} through a kinetic assay. Since the PLC_{Bc} analogues were structurally similar to the natural substrate phosphatidylcholine, it was expected that they would interact with PLC_{Bc} in the same manner as the natural substrate. In fact, all of the non-hydrolyzable substrate-based analogues thus far prepared in the Martin group, including phosphorodithioate **1.38g** and phosphonate **1.38c**, were all shown to be competitive inhibitors by the analysis of Lineweaver-Burk (double-reciprocal) plots.[87] X-ray diffraction data of complexes of PLC_{Bc} and mutant D55N with C6-cholinephosphonate **1.38c** and C5-choline phosphorodithioate **1.38h** revealed that these

analogs bound in the active site where the natural substrate would have otherwise bound,[80, 106] further suggesting that any inhibition observed due to those substrate-based analogues would be competitive. Therefore, it is reasonable to assume that the substrate-based analogues prepared in the prior sections of this chapter would be competitive inhibitors as well.

The kinetic assays were run under steady state conditions using a chromogenic assay previously developed for determining the kinetic parameters of PLC_{Bc} in this group. The assay quantitates the amount of the inorganic phosphate produced from the alkaline phosphatase catalyzed hydrolysis of phosphorylcholine, which is a hydrolyzed product of 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine by PLC_{Bc} (Figure 3.3).[76]

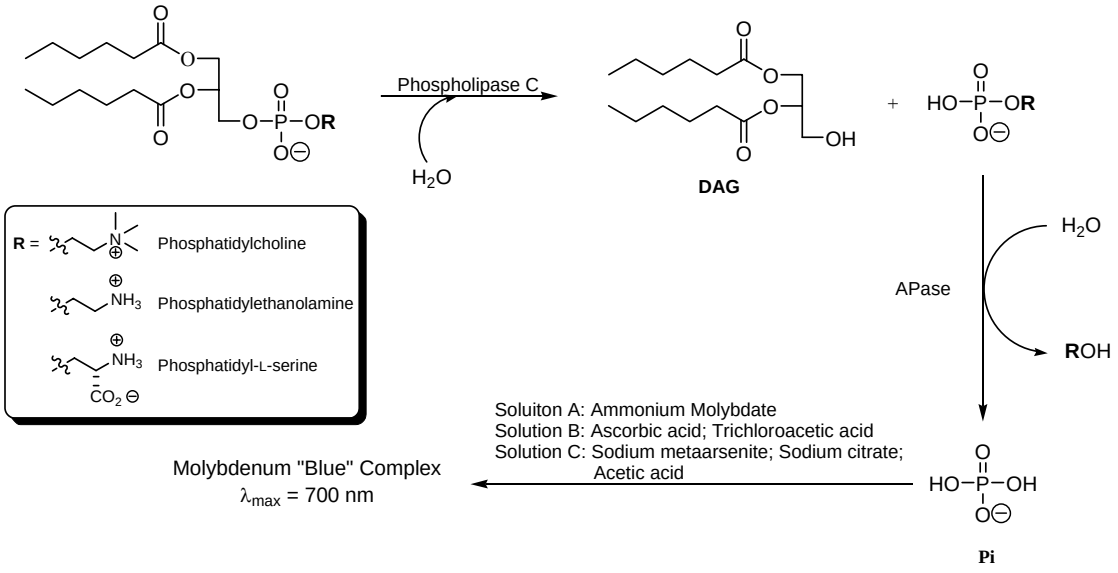


Figure 3.3. The chromogenic assay for determining the kinetic parameters of PLC_{Bc}

The initial velocities at different substrate concentrations were obtained from the intensity of the absorption of the phosphate-molybdenum complex in the assay, and the inhibition constants for all the analogues were extrapolated. Since the initial velocity was

measured under steady-state conditions, and the data was processed according to a simplified Michaelis-Mentor equation for competitive inhibition, it is beneficial to present a brief overview of related steady state kinetics concepts, mathematic equations and data processing methods in the upcoming sections.

3.3.1 Steady-state Kinetics and Competitive Inhibition

Kinetic assays were performed under steady-state conditions to simplify the mathematic equations necessary to process the data to obtain the kinetic parameters. In the steady state model, the enzyme-substrate complex ES can either dissociate into unbound enzyme and free substrate or undergo chemical reactions releasing product and reform free enzyme (Figure 3.4**)**. The enzymatic reaction starts with a pre-steady-state (transient-state) build-up of enzyme-substrate complex concentration (typically a few milliseconds). Since the rates of both dissociation pathways are first-order in the concentration of ES, the rate of decomposition of ES complex catches up rapidly. If the substrate concentration is in large excess of the enzyme concentration, the enzyme-catalyzed reaction reaches a situation of steady state after a few turnovers where the concentration of intermediate ES stays constant usually until an appreciable amount of substrate is consumed (Figure 3.4). The establishment of steady state is typically completed almost instantaneously after the catalytic reaction is initiated.

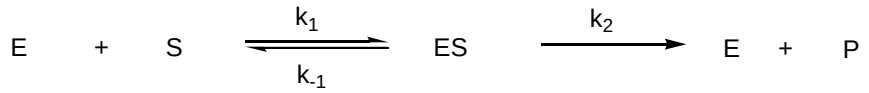


Figure 3.4: Simplified scheme for a one substrate-one product enzymatic reaction

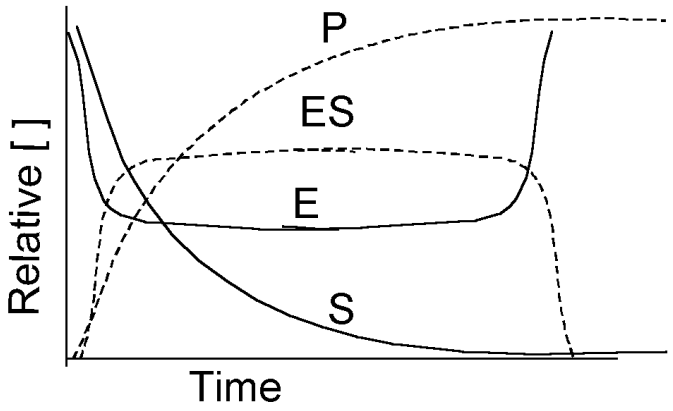


Figure 3.5: Concentration of major components in an enzymatic reaction.

Though the steady state can last for a period of time in a kinetic assay, only the initial velocities are typically measured. The concentration of the product at the time of measurement is thus practically negligible, which avoids the complications due to product inhibition. Another assumption is that the uncatalyzed chemical reaction is much slower than enzymatic catalyzed reaction so that its contribution to the overall product formation can be ignored. Briggs and Haldane in 1925[296] applied the steady-state approximation (Eq. 3.1) and mass balance (Eq. 3.2) to the kinetic scheme shown in Figure 3.4, provided a theoretical rationalization of the previously empirical Michaelis-Menten equation (Eq. 3.3).[297]

$$\text{Steady state assumption} \quad \frac{d[ES]}{dt} = k_1[E][S] - k_{-1}[ES] - k_2[ES] = 0 \quad (3.1)$$

$$\text{Mass balance} \quad E_0 = E + ES \quad (3.2)$$

$$v = \frac{k_2 E_0 S}{\frac{k_{-1} + k_2}{k_1} + S} = V_{\max} \frac{S}{K_M + S} \quad (3.3)$$

$$\text{Where} \quad K_M = \frac{k_{-1} + k_2}{k_1}, \quad V_{\max} = k_2 E_0$$

Initial velocity $v_0 = (\frac{dP}{dt})_{t=0} = k_{cat}[ES] = V_{\max}\frac{S}{K_M + S}$ (3.4)

Lineweaver-Burk plot (double reciprocal) $\frac{1}{v} = \frac{1}{V_{\max}} + \frac{K_M}{V_{\max}S}$ (3.5)

Abbreviations

E_0: enzyme concentration when t = 0, ie: total enzyme concentration

E: free enzyme concentration

S: free substrate concentration

I: free inhibitor concentration

I_0: total inhibitor concentration.

ES: enzyme-substrate complex concentration

EI: enzyme-inhibitor complex concentration

v: observed velocity of the enzymatic reaction

k: rate constant

V_{max}: maximum velocity for the enzymatic reaction, $v_{s\to\infty}$

K_M: equilibrium constant related to Michaelis-Menten kinetics

K_M^{app}: apparent Michaelis constant

K_i: inhibition constant

K_S: Dissociation constant for enzyme-substrate complex

Experimental data are traditionally analyzed by linear regression or more recently by computer assisted non-linear regression. The classical linear fitting approaches, Lineweaver-Burk[298] is simply an algebra conversion of the general Michaelis-Menten

equation to yield straight lines with with the slope being $\frac{K_M}{V_{\max}}$ and the Y-intercept being $\frac{1}{V_{\max}}$.

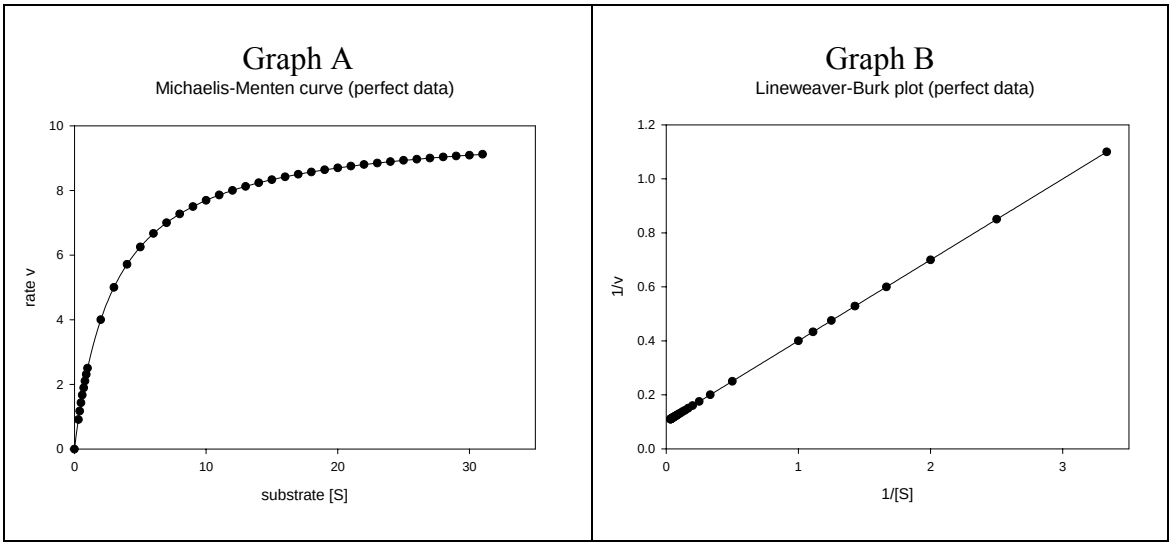


Figure 3.6: Michaelis-Menten curve and linearized data processing plot

The kinetics of the enzymatic reaction changes when compounds capable of interacting with the enzyme are present. The rest of this section will briefly discuss competitive inhibition since all the phospholipid analogs thus far prepared in the Martin group are competitive inhibitors.

By definition, a competitive inhibitor binds reversibly to the enzyme active site in place of the substrate, so the binding of the inhibitor excludes the binding of the substrate. This type of competitive inhibitor almost always bears some close structural and chemical similarity to the natural substrate of the enzyme. Alternatively, the inhibitor may bear no resemblance to the substrate and simply blocks the entry to the active site so that the substrate cannot enter. Another type of competitive inhibitor is one that binds to a

different site of the enzyme causing a conformation change in the enzyme rendering the active site unable to bind the natural substrate. Similarly, prior binding of the substrate to the active site causes a change in the inhibitor site preventing the inhibitor from binding. In either case, the binding to inhibitor and substrate are mutually exclusive, so it is impossible for both to bind to the enzyme at the same time. Mathematic deduction using the steady state assumption (Eq. 3.9), mass balance of the enzyme-containing species (Eq. 3.10) and inhibition constant equation (Eq. 3.11) would then give the competitive inhibition equation (Eq. 3.12).

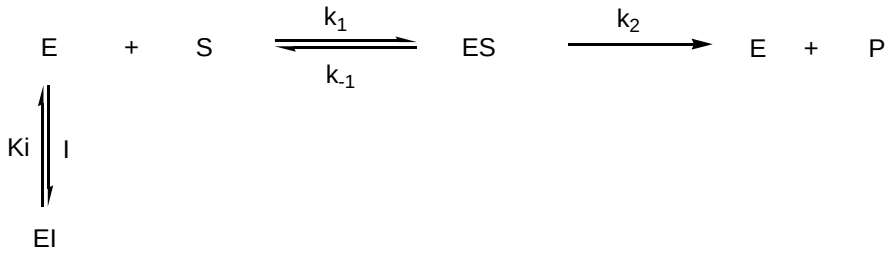


Figure 3.7: Simplified scheme for a one substrate-one product enzymatic reaction with a competitive inhibitor

Steady state assumption $\frac{d[ES]}{dt} = k_1[E][S] - k_{-1}[ES] - k_2[ES] = 0$ (3.9)

Mass balance $E_0 = E + ES + EI$ (3.10)

Inhibition constant $K_i = \frac{[I][E]}{[EI]}$ (3.11)

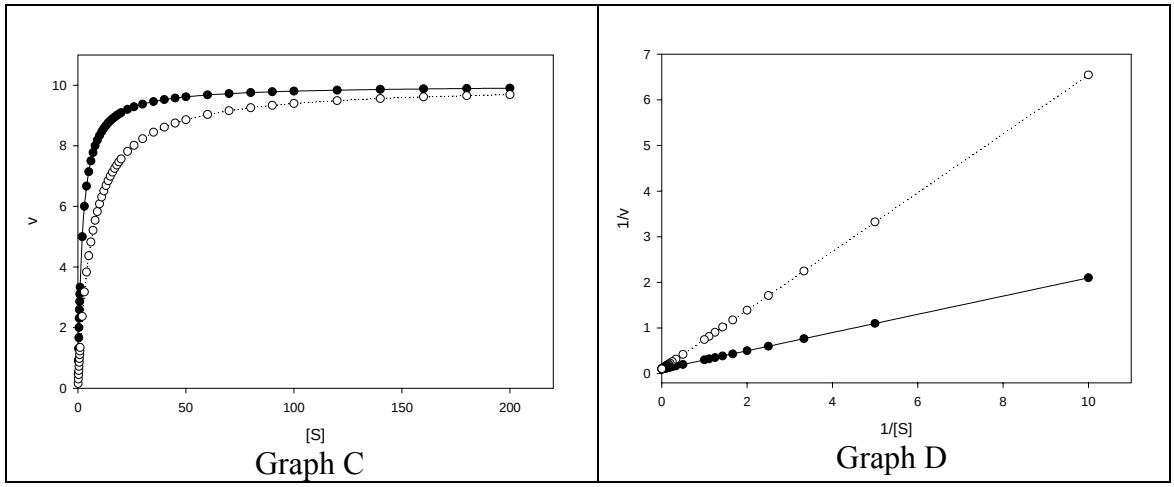
Competitive inhibition rate equation $v = V_{\max} \frac{S}{K_M(1 + \frac{I}{K_i}) + S}$ (3.12)

When $I_0 > E_0$, then $I \approx I_0$, then $v = V_{\max} \frac{S}{K_M(1 + \frac{I_0}{K_i}) + S}$ (3.13)

Define $K_M^{app} = K_M(1 + \frac{I_0}{K_i})$ (3.7)

$$\text{Therefore } \frac{K_M^{app}}{K_M} = 1 + \frac{I_0}{K_i} \Rightarrow K_i = \frac{I_0}{\frac{K_M^{app}}{K_M} - 1} = \frac{I_0}{\frac{V_{\max} / K_M^{app}}{V_{\max} / K_M} - 1} = \frac{I_0}{\frac{k_{cat} / K_M^{app}}{k_{cat} / K_M} - 1} \quad (3.14)$$

It is easily recognizable from the Michaelis-Menten plot that the inhibitor substantially reduces enzyme velocity at low substrate concentrations, but it does not appreciably alter velocity at very high substrate concentrations and V_{max} remains unchanged.



Graph C Graph D

Figure 3.8: Michaelis-Menten and Lineweaver-Burk plot for competitive inhibitors Graph C: Michaelis-Menten curve using perfect data. upper: control assay; lower: inhibition assay; Graph D: Lineweaver-Burk plot. upper: control assay; lower: inhibition assay

3.3.2 Modification To the Kinetic Analysis

The computer-assisted non-linear regression using the Michaelis-Menten equation was used in our group to obtain both K_M and K_{cat}. However, it has become a problem to establish accurate kinetic parameters when PLC_{Bc} mutants or inhibitors were used in the current project.[299] The intrinsic problems were the larger K_M or K_M^{app} and the tendency

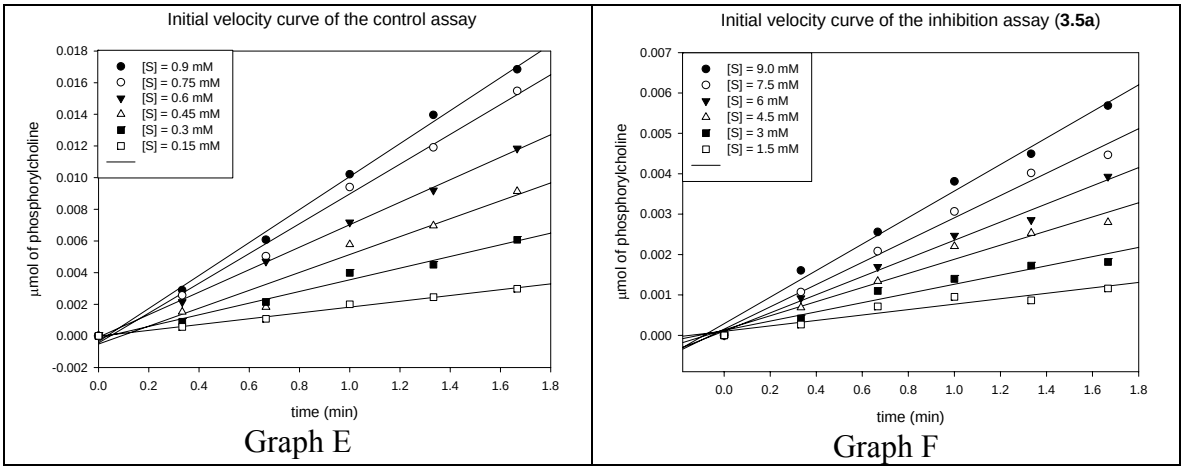
of the phospholipid substrate to form aggregates at higher concentration. Most of the mutants we obtained were inferior to the wild type phospholipase C with regard to catalytic efficiencies, so they had higher K_Ms for substrates. The Michaelis-Menten curves remained nearly linear at concentrations close to the critical micelle concentration (CMC) of the substrate C6PC **1.38a**. Hence the V_{max} thus obtained from non-linear regression bore a large error. The same problem existed with the inhibition assay as K_M^{app} is larger than K_s. Increasing the substrate concentration further was not an option since the kinetics of the hydrolysis changes at substrate concentrations above CMC, and significant acceleration of hydrolysis was reported.[300] Theoretically, we could use phospholipids with higher CMC's in the kinetic assays; unfortunately, those are generally worse substrates with higher K_Ms.

$$\text{Michaelis-Menten equation} \quad v = V_{\max} \frac{S}{K_M + S}$$

$$\text{if} \quad S << K_M \quad \text{then} \quad v \approx \frac{V_{\max}}{K_M} S = \frac{k_{cat} E_0}{K_M} S$$

Consequently, the data were collected and processed as described below. The assay was performed at lower substrate concentrations, and consequently higher concentrations of enzyme were sometimes used to obtain a normal response. Under these conditions, the Michaelis-Menten equation was linearized with the slope being $\frac{k_{cat} E_0}{K_M}$. The sacrifice here was neither K_{cat} nor K_M could be obtained individually; however, the value of $\frac{k_{cat}}{K_M}$ alone was sufficient to derive inhibition constant K_i (Eq. 3.14), the kinetic parameter of interest. An illustrative example is shown in Figure 3.9**.** The inhibition

constant was obtained from the ratio of the slopes of Michaelis-Menten plots at low substrate concentrations.



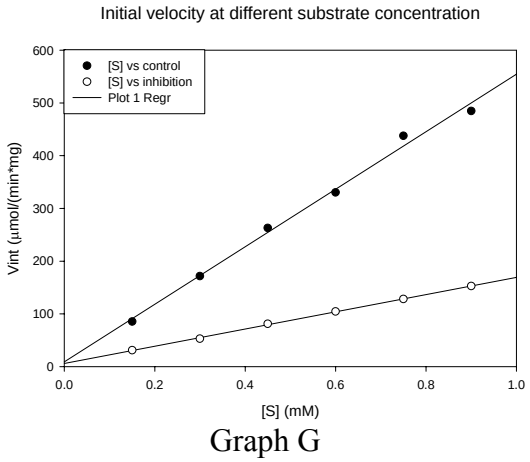


Figure 3.9: Initial Velocity Curve and Michaelis-Menten Plot at Low Substrate Concentration. Graph E: Initial velocity curve of the control assay (experimental data); Graph F: Initial velocity curve of the inhibition assay in the presence of **3.5a** (2.55 mM) (experimental data); Graph G: Michaelis-Menten plot at low substrate concentration.

3.3.3 Kinetic Evaluation of Water-Soluble Phospholipids

The potencies of the phospholipid analogues **3.34-3.36**, **3.48** and **3.53** as inhibitors of PLC_{Bc} were determined,[284] and most of the synthetic analogues were found to be inhibitors. The K_i and solubility of each analogue are shown in Table 3.2.

Table 3.2: Results of Kinetic and Solubility Studies on Phospholipid Analogues

Phospholipid	Solubility	K_i	Enzyme
C5-phosphonatePE **3.53b**	7.7 mM	No inhibition[#]	PLC_{Wt}
C6-phosphonatePE **3.53c**	280 mM	No inhibition[#]	PLC_{Wt}
C6-phosphonatePS **3.48**	6.8 mM	No inhibition[#]	PLC_{Wt}
C6-hydroxydithioPE **3.4a**	3.0 mM	0.17 mM	PLC_{Wt}
C6-hydroxydithioPC **3.3a**	37 mM	1.0 mM	PLC_{Wt}
C8-hydroxydithioPC **3.3b**	4.5 mM	2.6 mM*	PLC_{Wt}
C10-hydroxydithioPC **3.3d**	0.50 mM	0.04 mM	PLC_{Wt}
C6-hydroxydithioPS **3.5a**	very soluble	1.2 mM	PLC_{Wt}
C8-hydroxydithioPS **3.5b**	6.3mM	1.4 mM*	PLC_{Wt}

K_i values are average of two or more assays except as noted otherwise, *Values are from a single kinetic assay; [#]No inhibition observed at maximum solubility;

Examination of the results in Table 3.2 revealed some useful trends between inhibitor structure and potency. As expected, the short-chain **3.53b** was sufficiently soluble in water; however, no inhibition was observed at its maximum solubility. In fact, none of the phosphonate analogs inhibited PLC_{Bc} at their maximum solubilities. The dithiophosphatidylethanolamine **3.4a** and dithiophosphatidyl-L-serine **3.5a**, which were congeners of dithiophosphatidylcholine **3.3a**, were good inhibitors. The C6 dithiophosphatidylcholine **3.3a** and -L-serine **3.5a** and their C8 analogs **3.3b** and **3.5b** were about equipotent, while further increasing the length of the acyl side chains produced a more potent and less soluble C10 analog **3.3d**. Current data also suggested

that choline-, ethanolamine- and L-serine- derived phospholipids analogs have similar K_is with PS being the most soluble and PE the least soluble, which is expected considering the relatively low selectivity of PLC_{Bc} towards its natural substrates.

3.4 CRYSTALLIZATION STUDIES[301]

Experiments involving random mutagenesis of PC-PLC_{Bc} demonstrated that randomly changing the three amino acids residues E4, Y56, and F66 afforded variants with altered substrate specificities.[282] Recently, Antikainen and colleagues discovered that mutating Y56 to threonine changed the selectivity of wild type from PC to PE, while mutants E4G and E4Q were found to be specific for PS (Figure 3.10).[281] These mutants exhibited an impressive catalytic activity for their preferred phospholipids, about 2-4 times higher than the wild type. The fact that these dramatic changes in substrate specificity arose from a single amino acid mutation makes them interesting candidates for structural studies.

Obtaining crystal structures of the Y56T, E4G, and E4Q mutants complexed with analogs similar to their preferred substrates, PE and PS, respectively, will provide insight into the structural basis for the selectivity of these mutants. The structure of the unbound forms of E4G, E4Q and Y56T mutants were obtained and solved by Benfield[301] and found to be nearly superimposable to that of wild type. Many attempts were made to acquire crystal of complexes of these mutants of PLC_{Bc} with phospholipid analogs. High ligand occupancy was never achieved using phosphorodithioates, ω-hydroxy-phosphorodithioates **3.3**-**3.5** and phosphonate-PE **3.53**. Aaron Benfield successfully obtained complexes of phosphonate-PS **3.48** with E4G, E4Q and wild type and solved the structures.[301]

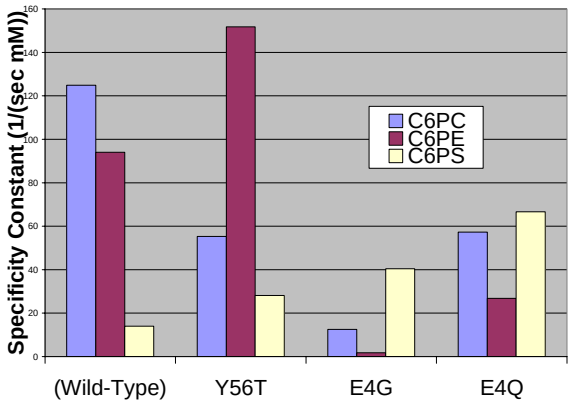


Figure 3.10: Catalytic Activity (Kcat/Km) of Wild-Type PC-PLC$_{Bc}$ and Mutants E4G, E4Q, and Y56T Towards PC, PE & PC.[281]

These three-dimensional structures are almost identical in that the backbone atoms align with an rmsd of 0.09 Å for E4G and E4Q complexed with phosphonate-PS **3.48**. However, these mutants bind the PS analogue in a very *different* manner than wild-type PC-PLC$_{Bc}$ does a PC analogue. Both structures reveal that a large conformational change involving the first 15 N-terminal residues occurred upon binding of the substrate analogue **3.48.** One of the zinc ions appears to have been lost. The phosphonate and fatty acid tails are located in the vicinity where Zn3 and Trp1 were found in the wt-PLC$_{Bc}$-PC structure.[80] The non-bridging oxygen atoms of the phosphodiester appear to contact Zn1, D55, His118, and Asp122 through electrostatic forces and hydrogen bonds. Additionally, the carboxyl group of the serine head group interacts with Zn2, Glu146, and His128. The zinc-binding pattern is very different from the wt-PLC$_{Bc}$-PC structure, where all three zincs are in close contact with the non-bridging oxygen atoms of the phosphodiester. There also appears to be a π-cation interaction between the amine nitrogen of the serine head group and Phe66 (Figure 3.11 and 3.12).[301]

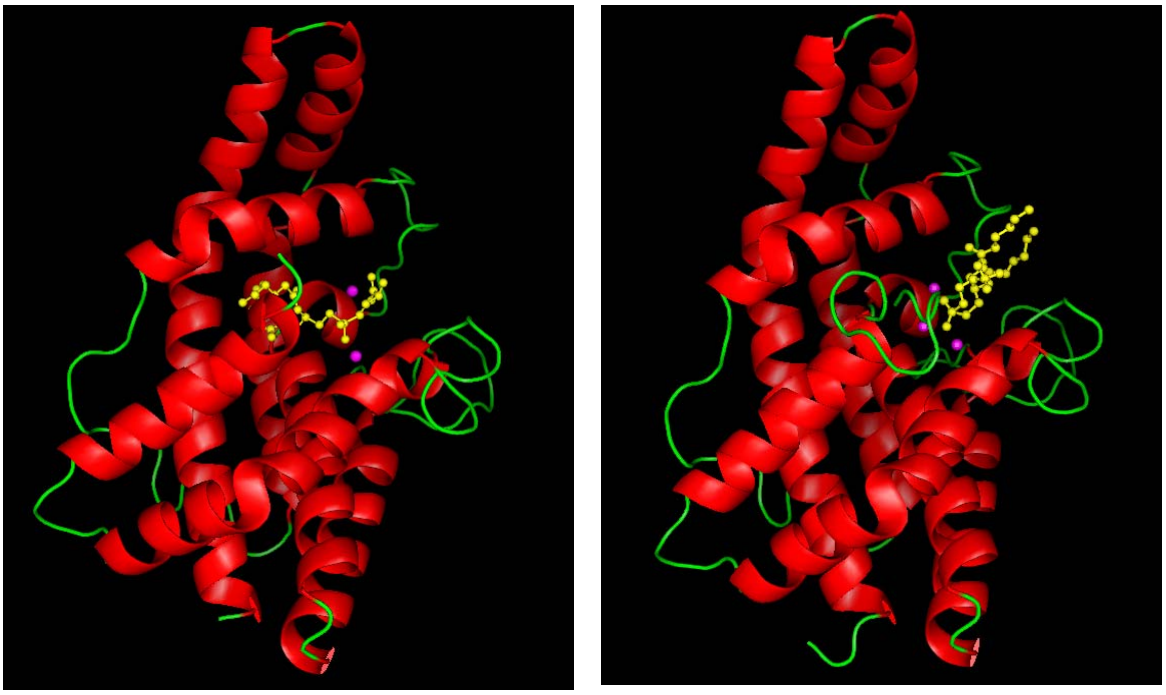
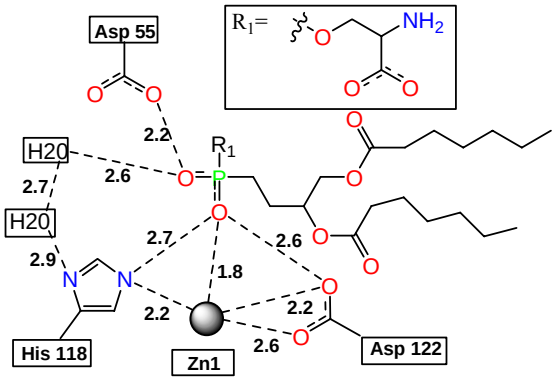


Figure 3.11: Side by side comparison of PLC_{wt}-PS and PLC_{we}-PC complexes. On the left: structure of PLC_{wt}-PS; on the right: structure of PLC_{wt}-PC



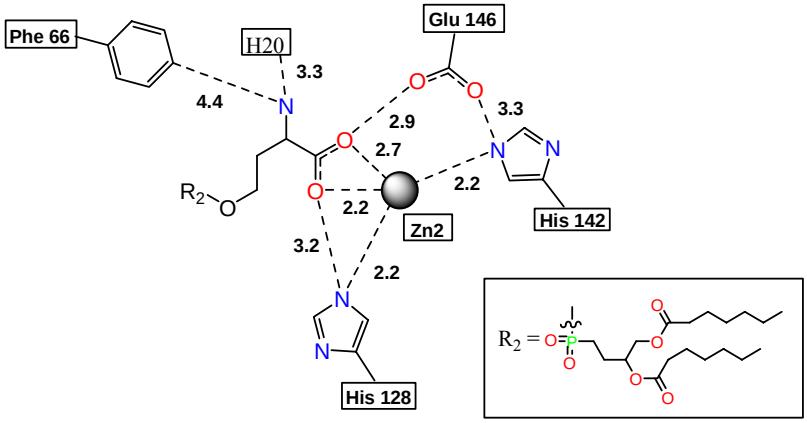


Figure 3.12: Interactions Between PLC mutant E4Q and **3.48**. Top: Interactions Between L-serine Phosphonate **3.48** and PLC (R1= Serine); Bottom: Interactions Between Serine Head Group and the PLC mutant, E4G.

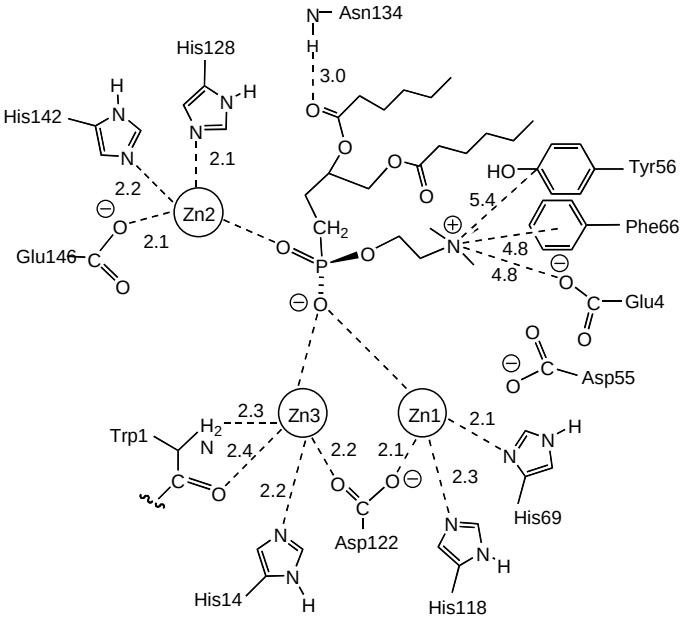


Figure 3.13: Cartoon representation of selected active site interactions in the structure of wt PLC_{Bc} bound to cholinephosphonate analog. Distances between atoms are shown in Ångstroms.

The observation that the PS analogue **3.48** binds these mutants and wild type in a very different manner as compared to the PC analogue **1.38c** suggests the enzyme could hydrolyze phosphatidylserine in a different fashion from phosphatidylcholine. Both mutants (E4G and E4Q) and wild type bind the PS analogue with only two zinc atoms. Only one of the two zinc atoms makes contact to one non-bridging oxygen atom of the phosphodiester, while the other involves in static interaction with the serine carboxyl group. Wild-type PC-PLC$_{Bc}$ utilizes all three zinc atoms to bind both non-bridging oxygen atoms of the phosphodiester of PC. It is suggested these three zinc atoms could play roles in activating the nucleophile water together with Asp 55, stabilizing the pentacovalent intermediate and stabilizing the leaving alkoxide as the general acid. However, in the crystal structure of PS analogue **3.48** with PS selective mutants E4G and E4Q and wild type, only one zinc ion within their active sites can potentially be involved in the catalysis. While a mechanism involving a single zinc ion could be proposed,[86, 103] extensive kinetic experiments beyond the scope of this project would be required to validate the proposal. Alternatively, these structures presented above could merely represent how these proteins bind the PS analogue **3.48** under certain conditions. It is possible that the PS binds in a very different manner *in vivo*. Without further experimental proof, an unambiguous conclusion cannot be drawn at this point.

3.5 CONCLUSION

A number of water-soluble non-hydrolyzable substrate analogs were prepared, including ω-hydroxy phosphorodithioates and phosphonates of choline, ethanolamine and L-serine. The phosphorodithioates function by chelation to one or more of the zinc ions at the active site of the enzyme. Kinetic assays reveal that ω-hydroxy phosphorodithioates in general are good inhibitors with decent aqueous solubility. Ethanolamine and L-serine

derivatives of phosphonates, however, failed to inhibit PLC_{Bc} at their maximum solubility. The three-dimensional structures of phosphonate-PS **3.48** with E4G, E4Q and wild type were solved by Benfield and revealed that these mutants bind the PS analogue in a very *different* manner than wild-type PC-PLC_{Bc} does a PC analogue. The structural difference again raised a number of questions about our understanding of phospholipase, presenting us with more challenges and opportunities in the future work.

Chapter 4: Studies Towards the Synthesis of 5-Hydroxyaloin A

4.1 THE CHEMISTRY AND BIOLOGY OF ALOE NATURAL PRODUCTS

Aloe is a group of tropical/subtropical plants that is characterized by lance shaped leaves with jagged edges and sharp points. It consists of over 400 different species, most native to the dry regions of Africa with most species found in southern and eastern Africa and the island of Madagascar. Most sources have placed Aloe in the Lily family (Liliaceae), which includes garlic, onion, and asparagus. Aloes vary in size ranging from little one-inch miniatures to species that can reach height of 60–90 cm long and 5–10 cm across the base.[302]

Figure 4.1: The economically most important aloe species: *Aloe vera* (*Aloe barbadensis*) (Pictures are used with consents from the photographers)

The term aloe is derived from the Arabic word “alloeh”, which means a shining bitter substance in reference to the exudate.[303] Aloes have been used therapeutically for the treatment of constipation, burns, cuts, rashes and skin disorders since biblical times and probably long before.[304] The leaves of Aloe yield two medicinal products – a thick, mucilaginous, colorless gel from the leaf and a bitter exudate from the outer layer of the leave. The gel is a typical ingredient in a range of healthcare, nutraceutical and cosmetic products, such as shampoos and skin care creams because of its moisturizing and soothing properties. Aloe gel has been reported to enhance immunity, improve liver function, prevent asthma and act as an anti-inflammatory, anti-ulcer, anti-diabetes, anti-hypertension, anti-cancer and antibiotic agent.[304] The bitter exudates known as "bitter aloes" or "aloe drug" are used mainly as laxatives and as bittering agents in certain beverages.[303]

For centuries, the leaves of the Aloe plant were used for its therapeutic properties without any insight of the chemical and biological basis for the wide spectrum of biological activities. Only in the last several decades did scientists begin a quest to understand the significant chemical ingredients that are responsible for the varied biological activities.

The leaf pulp (inner gel) contains more than 98% water, and its alcohol-insoluble portion has a high content of polysaccharides, monosaccharides, uronic acid and a few enzymes. Most researchers attribute the beneficial medicinal properties of the inner gel to the polysaccharide component. Significant variations in the composition of pulp polysaccharide species were reported in early studies, and a variety of polysaccharides have been detected or isolated from the pulp, including mannan, galactan, arabinan, arabinorhamnogalactan, pectic substance and glucuronic acid-containing polysaccharide. The reason for the variance in composition was probably related to seasonal change

and/or different geographic locations of the samples. The gel most likely has more than one active constituent, which may be addressing different biological properties.

The exudates are largely phenolic in nature. More than 130 secondary metabolites have been isolated from the leaves and roots of Aloe, including many alkaloids, anthraquinones, pre-anthraquinones, anthrones, chromones, flavonoids, coumarins and pyrones. The UV spectrum can be used to get a rapid idea of the different classes of compounds present in the methanol extract of leaf exudate and establish the chemical profiles in Aloe, if the analysis is performed using a modern reversed phase HPLC equipped with photodiode array detector.[303]

Anthrones are the most important secondary metabolites of all the classes of compounds present in Aloe. The gastrointestinal effects of the leaf exudates, particularly the laxative properties, are associated with the anthraquinone contents of the plant. The representative members of this class are aloin A (**4.1a**) and B (**4.1b**), which are collectively known as barbaloin. Aloin A/B are two β-*C*-anthracyl glucosides that are epimeric at *C*-10 where the glucose moiety is attached to the anthrone core. The absolute configuration of aloin B is established to be (10R, 1'S), and the epimeric orientation (i.e. 10S, 1'S) has been assigned to aloin A. These compounds are believed to be the primary factor for the bitter and purgative properties of the commercial aloe drug.

Grün and Franz have studied the biosynthesis of aloin, and they demonstrated that aloin B (**4.1b**) was formed by attachment of glucose to the aloe-emodin anthrone **4.2**, which in turn appeared to arise biosynthetically from a polyketide precursor. They further established that aloin B (**4.1b**) is the true natural product, which upon standing gradually epimerizes to give the artifact aloin A (**4.1a**).[303]

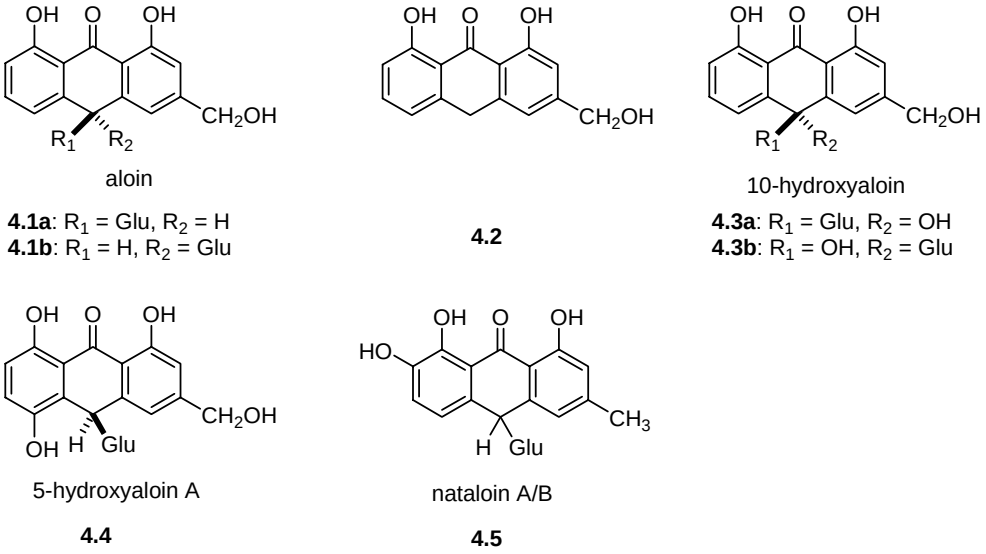


Figure 4.2: Representative anthrones in Aloe

On the other hand, 5-hydroxyaloin A (**4.4)**, which is isolated from leaf exudates of many Aloe plants, including *Aloe microstigma*, *Aloe broomii*, and Aloe marlothiionly, is known only in the more stable A-configuration (10R, 1'S), thus being the only known anthrone-*C*-glycoside that has not been found as diastereomeric pair in plants.

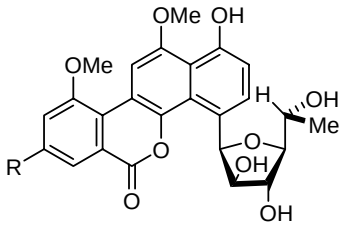
4.2 5-HYDROXYALOIN A

The aloin *C*-aryl glycosides possess 2-oxygenated sugars *para* to the central anthryl hydroxy functional group, and therefore they are technically classified as members of the Group I *C*-aryl glycoside family. However, Aloin *C*-aryl glycosides differ significantly from typical members of major classes of *C*-aryl glycosides, including Group I *C*-aryl glycosides in two aspects.

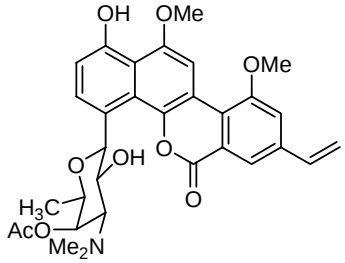
1. The point of the carbohydrate attachment to the aromatic core is different. In aloin *C*-aryl glycosides; the attachment is at the center of the anthrone ring, while all

other known naturally occurring *C*-aryl glycosides possess the carbohydrate moiety on the outermost ring of the aromatic system.

2. The nature of the carbohydrate moiety is different. The aloin *C*-aryl glycoside contains a 2-oxygenated carbohydrate, either a glucose or a derivative thereof; while most other known natural occurring *C*-aryl glycosides contain 2-deoxy sugars with only a few exceptions. Those exceptions all belong to the Group I *C*-aryl glycosides, such as gilvocarcins (**2.2a-c),** ravidomycin (**2.3**) and chrysomycin.



gilvocarcin M (**2.2a**): R = methyl
gilvocarcin V (**2.2b**): R = vinyl
gilvocarcin E (**2.2c**): R = ethyl



ravidimycin (**2.3**)

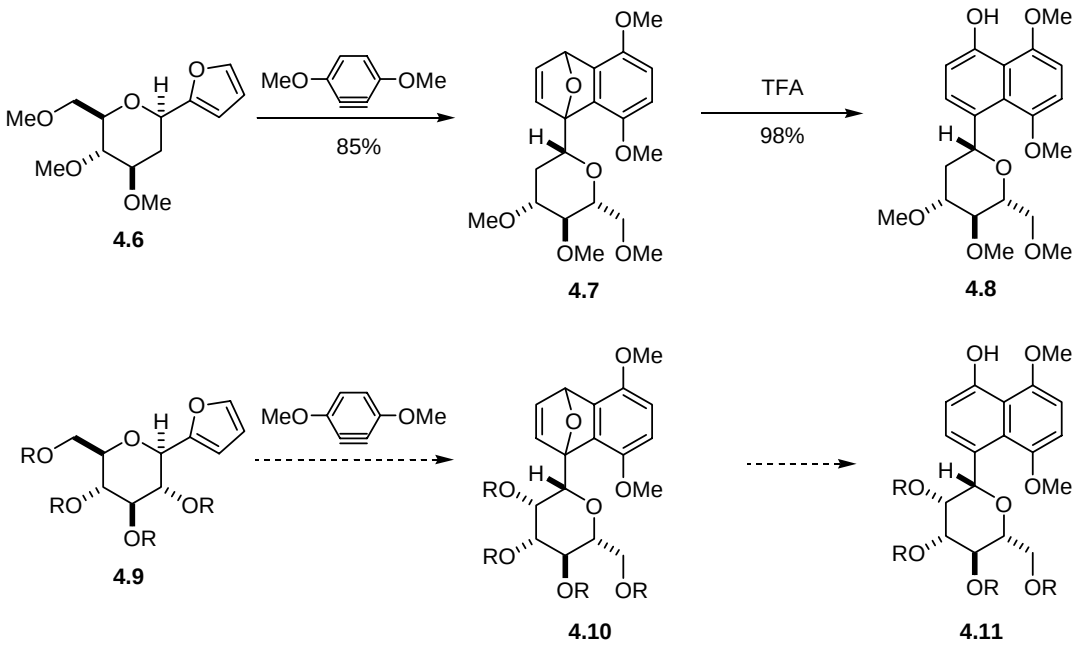
Figure 4.3: Group I *C*-aryl glycosides possessing 2-oxygenated carbohydrate moiety.

4.1.2 The First Generation Approach

We were interested in the synthesis of a representative aloin *C*-aryl glycoside using the tactics developed in our group to establish the feasibility of our unified approach to prepare the major classes of naturally occurring *C*-aryl glycosides. In our prior investigations, the more commonly occurring 2-deoxy sugars such as **4.6** were universally employed. Cycloaddition with benzyne followed by acid-catalyzed rearrangement then delivered glycosyl-substituted naphthol **4.8**. Hence the reactivity of a glycosyl furan with a 2-oxygenated carbohydrate moiety such as **4.9** had never been explored. Both the cycloaddition of **4.9** and the acid-catalyzed ring opening of **4.10** could

potentially be affected by the extra hydroxyl group on the carbohydrate moiety (Scheme 4.1).

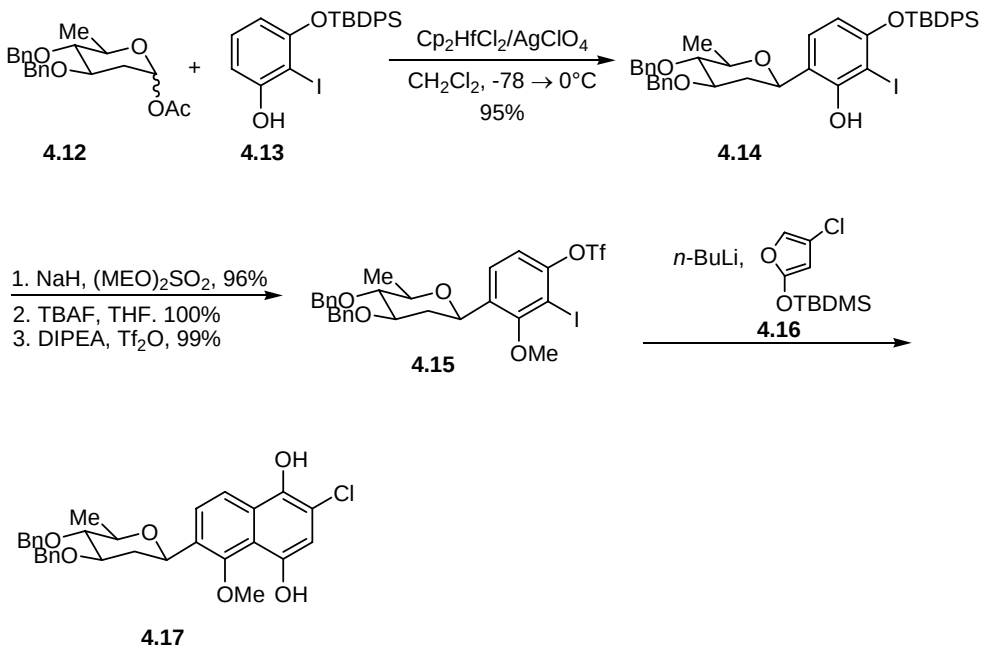
Scheme 4.1



We were particularly interested in the synthesis of Group I *C*-aryl glycosides, because of the advantage of our glycosyl furan/benzyne cycloaddition methodology in generating the 1,4-subitituion pattern of the glycosyl-substituted phenol. As discussed in the prior chapter, Suzuki developed a convergent approach that relied on regioselective cycloadditions of glycosyl-substituted α-alkoxybenzynes with alkoxyfurans.[171, 172] However, since the requisite glycosyl-substituted α-alkoxybenzynes were obtained from the *ortho* selective *O*→*C*-glycoside rearrangement, Group I *C*-aryl glycosides cannot be accessed directly. Hence Suzuki applied the *O*→*C*-glycoside rearrangement to a monoprotected resorcinol such as **4.13** to afford the 6-glycosyl resorcinol **4.14**, in which one phenolic hydroxyl was *ortho* to the carbohydrate moiety and the other *para* (Scheme

4.2). Subsequent benzyne generation eliminated the unwanted *ortho* phenolic hydroxyl. This tactic allowed Suzuki to circumvent the obstacle inherent in the *ortho* selective *O*→*C*-glycoside rearrangement, and his group later completed the total syntheses of three representative Group I *C*-aryl glycosides, gilvocarcin M (**2.2a**),[146] gilvocarcin V (**2.2b**)[146] and ravidomycin (**2.3**).[173, 180] Our benzyne cycloaddition/ring opening methodology, however, allows direct access to the Group I *C*-aryl glycosides. Therefore we were expecting to demonstrate the effectiveness of our methodology in the synthesis of naturally occurring Group I *C*-aryl glycosides.

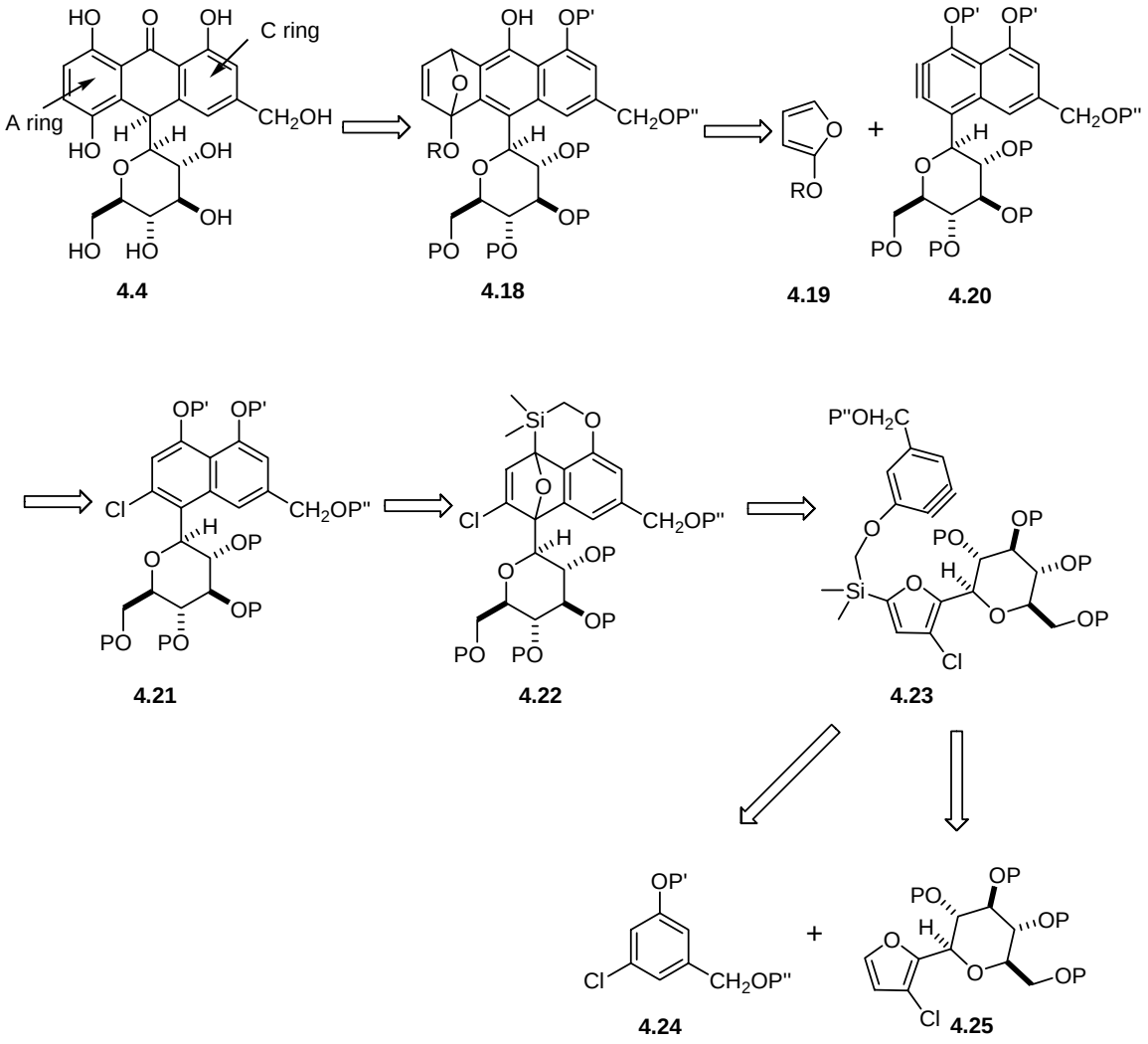
Scheme 4.2



The distinctive architecture of the aloin *C*-aryl glycoside with a 2-oxygenated carbohydrate attached at the center of a polyphenolic aromatic core provides an excellent opportunity to demonstrate the strength and utility of our newly developed methodology. The representative 5-hydroxyaloin A (**4.4**) was chosen as the synthetic target. It was

envisioned it could be synthesized through annulating new aromatic rings onto an existing ring in two directions, Our approach, which is illustrated in Scheme 4.3, highlights two consecutive furan-benzyne [4+2] cycloadditions. These are a glycosyl-furan/benzyne cycloaddition and a regioselective glycosyl-substituted α-alkoxybenzyne/alkoxyfuran cycloaddition. Successful completion of this route relies on the ability to generate a benzyne intermediate from a naphthalene precursor **4.21**. Since the glycosyl-furan/benzyne cycloaddition has consistently given poor global regioselectivity, regiocontrol *via* a disposable silyl tether in the first cycloaddition is required to produce the correct regioisomer **4.22**. The symmetric disposition of substitution of A ring however renders the control of regiochemistry in the second cycloaddition unnecessary. Thus, the three essential components are the siloxyfuran **4.19**, the *m*-chlorophenol **4.24** and 3-chloro-2-glycosylfuran **4.25**.

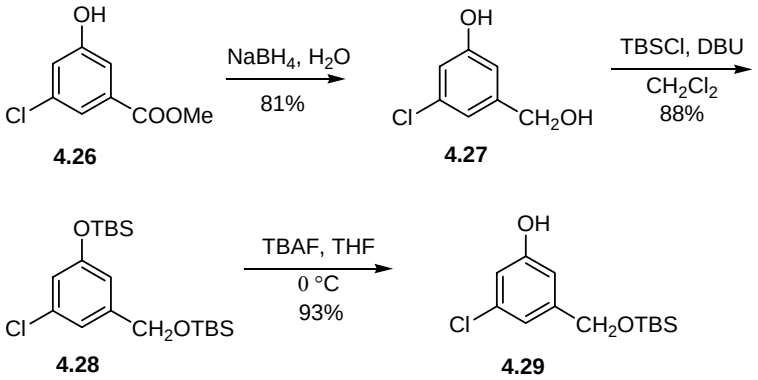
Scheme 4.3



The required phenol **4.29** was synthesized from the known methyl ester **4.26** (Scheme 4.4). Reduction of the ester **4.26** with DIBAL-H or aqueous sodium borohydride afforded the hydroxymethyl phenol **4.27**. Both hydroxyl groups of **4.27** were protected as their di-*tert*-butyldimethylsilyl ethers. Selective deprotection of bis-TBDMS ether **4.28** with tetrabutylammonium fluoride (TBAF) at low temperature afforded the requisite phenol **4.29**. Treatment of *m*-hydroxymethylphenol **4.27** with TBSCl/DMAP/ Et_3N[305] in

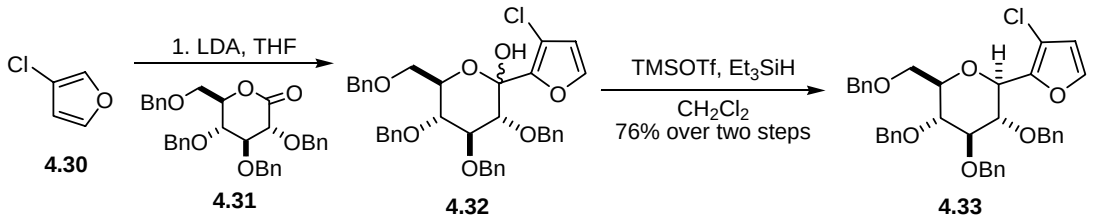
an attempt to achieve selective protection of the primary hydroxyl group in the presence of a phenolic hydroxyl resulted in protection of the phenolic hydroxyl group, instead of the benzylic alcohol.

Scheme 4.4



The synthesis of glycosyl furan **4.33** started from known 2,3,4,6-tetra-*O*-benzyl-D-glucopyranolactone (**4.31**)[306] and 3-chlorofuran (**4.30**)[307, 308] (Scheme 4.5). Regioselective α-lithiation of 3-chlorofuran (**4.30**) with LDA and subsequent addition of the resultant 2-lithio-3-chlorofuran to glucopyranolactone **4.31** afforded the hemiacetal **4.32**. Without purification, hemiacetal **4.32** was treated with trimethylsilyltriflate (TMSOTf) in the presence of triethylsilane, during which time oxocarbenium generation and regioselective hydride reduction ensued to give the β-furyl glucopyranose **4.33** predominantly[184] (Scheme 4.5). The β configuration of the anomeric carbon was evidenced by the large coupling constant (J = 10.5 Hz) of the anomeric proton. If desired, the small amount α-anomer may be conveniently removed through recrystallization of the mixture from hexanes/chloroform. The acidic $NaCNBH_3$, which was used to reduce the lactols possessing 2-deoxy sugars, failed to reduce **4.32** completely.[182]

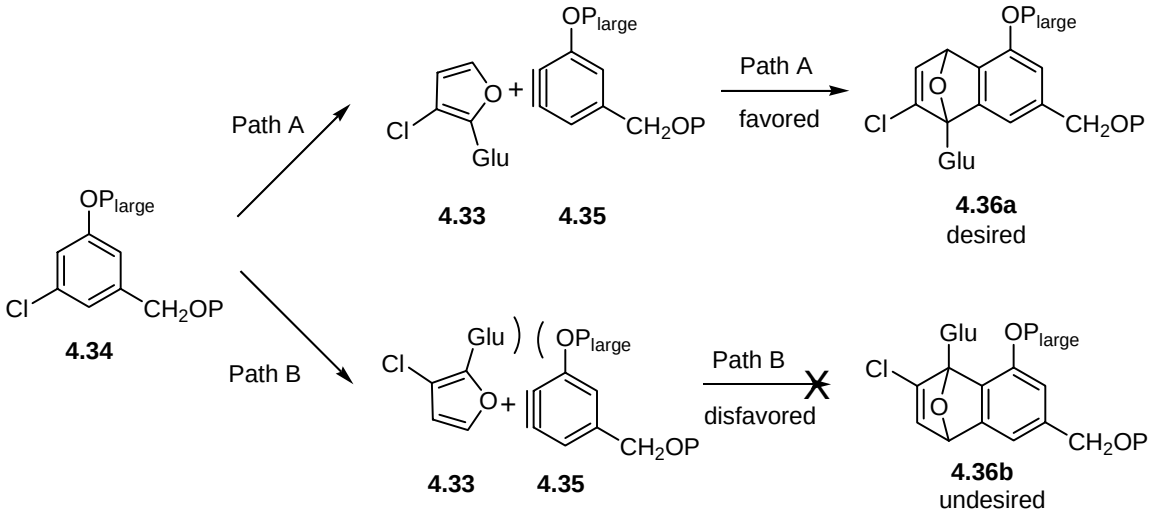
Scheme 4.5



Sterics to Control Regiochemistry

Prior to adopting the tethering strategy for effecting regiocontrol in glycosyl furan/benzyne cycloaddition, model studies were conducted to probe whether regiochemistry could be controlled based on steric factors. This tactic would have avoided multiple-step tethering and tether cleavage. We envisioned that a bulky protecting group, such as triphenylmethyl (trityl), might hinder the approach of 2-glycosylfuran **4.33**, and hence raise the activation energy associated with Path B. Hence the formation of the desired regioisomer **4.36a**, in which the carbohydrate moiety was further away from the bulky protecting group, would be favored (Scheme 4.6). We were however cautious about this strategy due to precedence in the literature of poor selectivity in intermolecular benzyne cycloadditions and our past failure in controlling regiochemistry based on steric factors as already discussed in section 2.32).[271, 272]

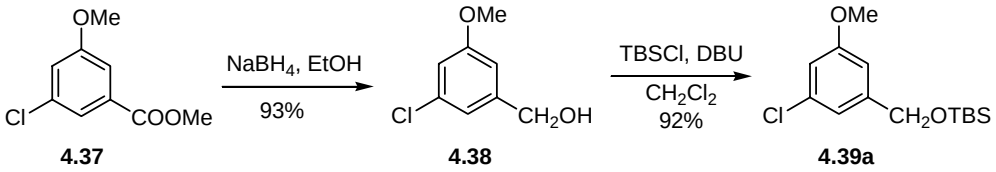
Scheme 4.6



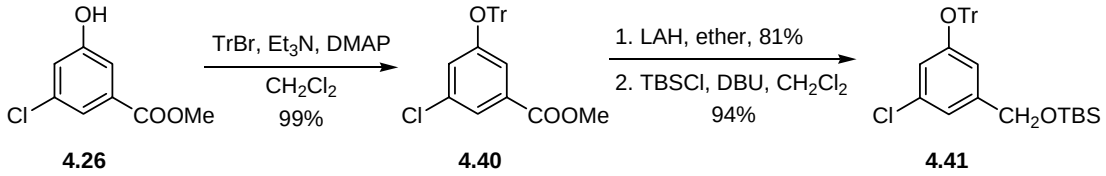
To test the viability of using steric effects to control regiochemistry in the cycloadditions, *m*-chlorophenol trityl ether **4.41** was prepared from the known phenol **4.26** (Scheme 4.8), and the related *m*-chloroanisole **4.39a** was made as the control compound (Scheme 4.7). We found that **4.39a** readily underwent metallation *ortho* to the methoxy group and chloride upon exposure to *s*-BuLi at low temperature. Subsequent warming up in the presence of furan or 2-glycosylfuran **4.33** led to the desired cycloadducts. In sharp contrast, the trityl ether **4.41** failed to undergo *ortho*-metallation using *n*-BuLi, *s*-BuLi or LDA and consequently there was no cycloaddition with glycosyl furan **4.33**. The very steric bulk imposed on the phenolic oxygen in order to achieve the regiocontrol in the cycloaddition step worked against us in the metallation step, depriving the phenolic oxygen of the capability of serving as an *ortho*-directing group. Although **4.41** could be altered to allow *ortho*-lithiation and subsequent benzyne generation, either by installing a secondary functional group such as a halide or by reducing the steric bulk

around the phenolic oxygen. The precedented failure in the literature promoted us to steer away from further efforts in this approach.[271, 272]

Scheme 4.7



Scheme 4.8

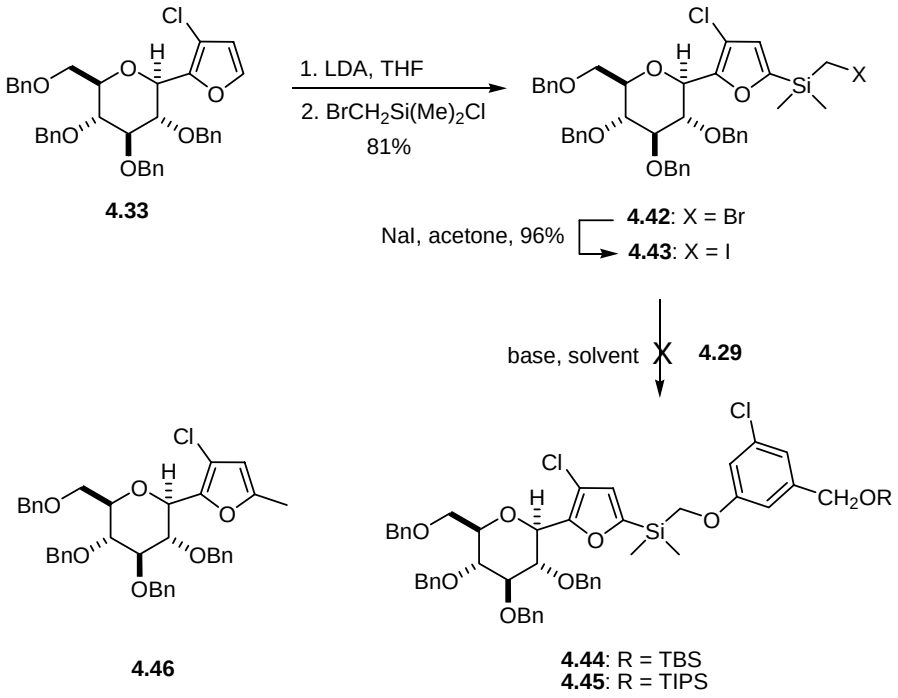


Tether to Control Regiochemistry-Williamson Etherification

In view of our inability to exploit steric effects to control regiochemistry in the [4+2] cycloaddition, we turned to a tether strategy. Thus a silyl tether was installed onto **4.33** using a method developed in the Martin group.[268] α-Lithiation of 3-chloro-2-glycosylfuran **4.33** with LDA followed by trapping the resultant lithiofuran with bromomethyldimthylsilyl chloride afforded the furylsilane **4.42**. Bromomethylsilane **4.42** and its corresponding iodide **4.43** were then subjected to Williamson etherification in an effort to attach the arene moiety and obtain the cycloaddition precursors **4.44** or **4.45**. Unfortunately, attempts to implement this strategy gave either no reaction or trace amounts of desired product **4.44** or **4.45** that was accompanied with significant quantities of **4.46**, which no longer possessed the silyl tether handle (Scheme 4.9). The formation of **4.46** was apparent with an upfield shift of furan proton by 0.7 ppm and the formation of a singlet at 2.2 ppm in the ^{1}H NMR spectrum. It was also found that the

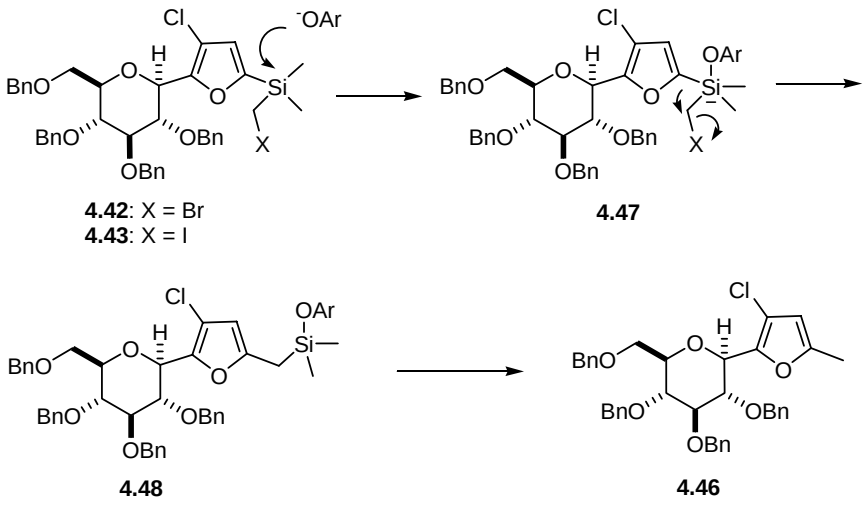
bromomethylsilane **4.42** was not stable to the standard Williamson etherification conditions that were used in our group to similar ethereal compounds.[268] Simply stirring **4.42** with Cs_2CO_3 and TBAI in acetone in the absence of phenol **4.29** cleanly afforded the desilylated by-product **4.46**.

Scheme 4.9



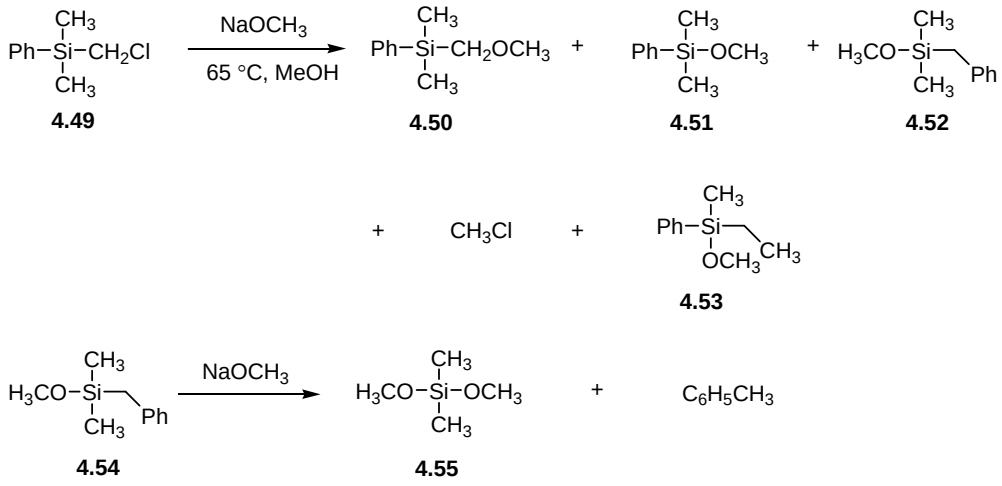
The main decomposition pathway responsible for the formation of **4.46** was presumably initiated by the nucleophilic attack on silicon rather than carbon. Subsequent aryl migration and desilylation, either through protodesilylation or a second nucleophilic attack followed by elimination, resulted in the formation of **4.46** (Scheme 4.10). This type of rearrangement has been reported several times in literature, but mostly with fluoride[309, 310] and alkoxide nucleophiles.[311-314]

Scheme 4.10



Shechter and Kreeger investigated the reaction of chloromethyl dimethyl-phenylsilane (**4.49**) with sodium methoxide at 65 ºC in methanol (Scheme 4.11).[313] The major product of the reaction was **4.50**, which was formed by displacement of chloride with methoxide. Small amounts of **4.51** and toluene, both of which were resulted from initial attack of methoxide on silicon were also isolated (Scheme 4.11). Addition of 18-crown-6 in trace amounts caused a shift from predominant methoxide attack on carbon to that on silicon and as a result a dramatic shift in the product distribution. Substituting potassium methoxide or cesium methoxide for sodium methoxide or switching solvent from methanol to dioxane had similar impact on the reaction pathway. The use of lithium, magnesium, or calcium methoxide in this reaction gave only recovered starting material. Shechter therefore concluded that the more separated and thus more nucleophilic methoxide ion preferentially attacked the silicon resulting in the observed side reactions.[313]

Scheme 4.11



In a separate study probing the effects of the steric bulk and nucleophilicity on the reaction of alkoxides (*t*-BuO$^-$, *i*-PrO$^-$ and MeO$^-$) with chloromethylvinylsilane in tetrahydrofuran, Shechter observed that as the bulk of the alkoxide increased, attack on silicon diminished. He proposed that attack on silicon to generate a pentavalent intermediate was a process that was more sensitive to steric hindrance. Therefore, the more bulky alkoxide ion preferentially attacked the less steri*C*-demanding carbon.[314]

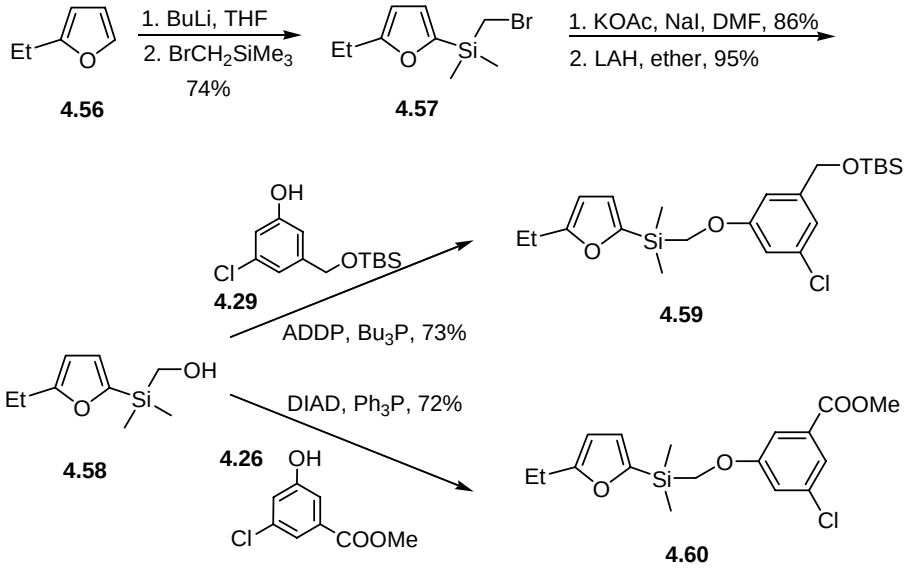
With these precedents in mind, attempts were made to optimize the etherification reaction by using a bulky protic solvent (*t*-butyl alcohol and *t*-amyl alcohol), and a pre-formed lithium or sodium salt of the phenol **4.29** in hopes that the nucleophilic attack on the less hindered carbon would prevail. However, all of these efforts, including the use of the less nucleophilic phenoxide from **4.26**, afford either mostly **4.46** or unreacted starting material.

Tether to Control Regiochemistry-Mitsunobu Etherification

An alternate means to activate the alcohol as a leaving group in order to link 3-chloro-2-glucosyl furan **4.33** and phenol **4.29** *via* a Mitsunobu reaction was developed. Toward this end, the model α-silanol **4.58** was chosen to probe the feasibility of this strategy. The α-silanol **4.58** was prepared by reduction of the corresponding acetate with lithium aluminum hydride (LAH), which in turn was prepared by direct displacement of the bromide of **4.57** with acetate. Gratifyingly, the Mitsunobu etherification between α-silanol **4.58** and phenols **4.29** and **4.26** proceeded smoothly to afford the desired products **4.59** and **4.60** in good yields (Scheme 4.12).

This success notwithstanding, etherification of α-silanol **4.62** proved to be problematic. The reaction did afford the desired product; however, the yield was rather poor, ranging from 20-30% (Scheme 4.13). Attempts to optimize the reaction by changing solvents, reaction temperature and order of addition resulted in only little improvement. It was initially thought that the phenolic hydroxyl was not acidic enough, so the more reactive combination-tributyl phosphine and ADDP (azodicarbonyl dipeperidine) was employed.[315] The yield, however, was not improved and in one case the yield actually dropped unexpectedly to 9%. Use of the more acidic phenol **4.26** was equally unsuccessful. NMR again revealed the formation of the desilylated product **4.46**, suggesting that nucleophilic attack on the silicon had taken place.

Scheme 4.12



Scheme 4.13

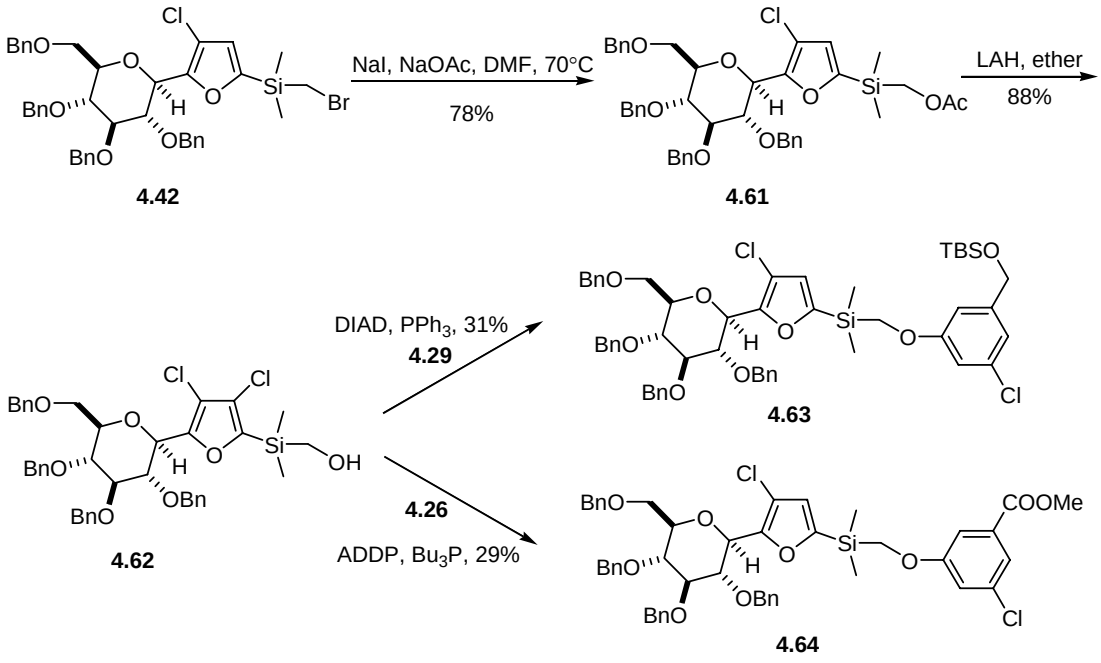
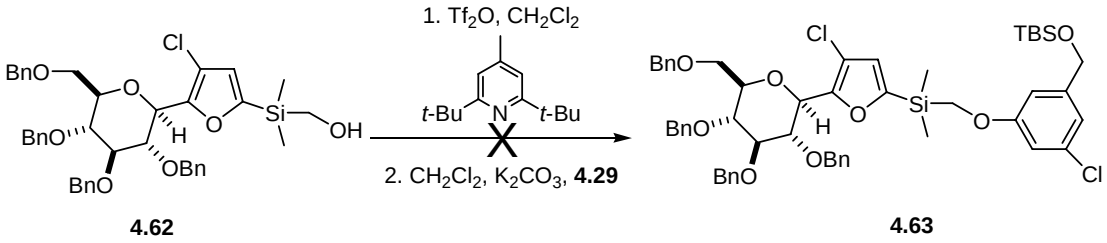


Table 4.1: Attempts in the Mitsunobu Etherification of **4.62**

Entry	Phenol	Other reagents	solvent	Temp.	yield
1	**4.29**	PPh_3, DIAD	toluene	rt	20%
2	**4.29**	PPh_3, DIAD	THF	rt	slow reaction
3	**4.29**	PPh_3, DIAD	CH_2Cl_2	rt	24%
4	**4.29**	PPh_3, DIAD	benzene	rt	20-31%
5	**4.29**	PPh_3, DIAD	benzene	0 °C	18%
6	**4.29**	PPh_3, DIAD	benzene	–15 °C	11%
7	**4.29**	PPh_3, DIAD	benzene	–78 °C	0%
8	**4.29**	PBu_3, ADDP	benzene	rt	9%
9	**4.26**	PPh_3, DIAD	toluene	rt	24%
10	**4.26**	PBu_3, ADDP	benzene	rt	22%
11	**4.26**	PBu_3, ADDP	THF	rt	10% (slow reaction)
12	**4.26**	PBu_3, ADDP	CH_2Cl_2	rt	29%

It was thought that a better leaving group would facilitate the second-order S_N2 substitution on the carbon, and subsequent displacement with phenoxide would then afford the desired cycloaddition precursor.[316] Hence α-silanol **4.62** was transformed into its triflate and allowed to react with phenol **4.29**. However, only trace amounts of the desired product **4.63** were observed from this one-pot reaction (Scheme 4.14). It was not clear whether the intermediate triflate had actually formed or the displacement failed to proceed.

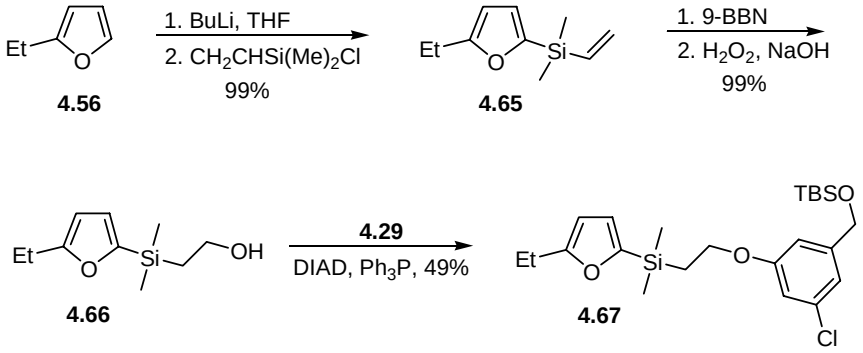
Scheme 4.14



4.62 **4.63**

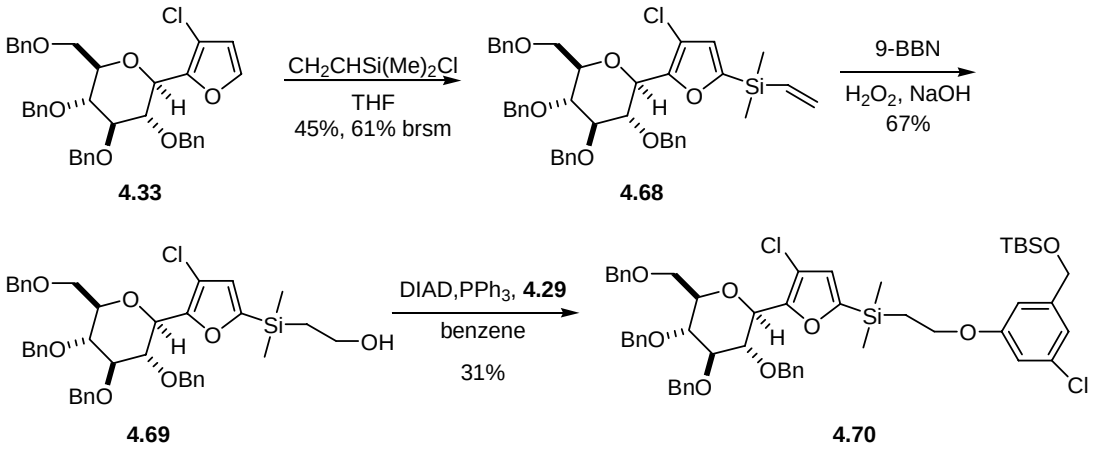
To get around the problem of the competing α-halosilane rearrangement initiated by nucleophilic attack on silicon, a strategy using a silyl tether containing an additional carbon atom that was developed by group member Dr. Sparks was adopted.[278] The model β-silanol **4.66** was prepared *via* oxidation/hydroboration of the corresponding vinylsilane **4.65**, which was obtained by the reaction of 2-ethyl-5-lithiofuran with dimethylvinylchlorosilane. When β-silanol **4.66** and phenol **4.29** were subjected to Mitsunobu etherification conditions, the desired product **4.67** was obtained in an unoptimized yield of 49% (Scheme 4.15).

Scheme 4.15



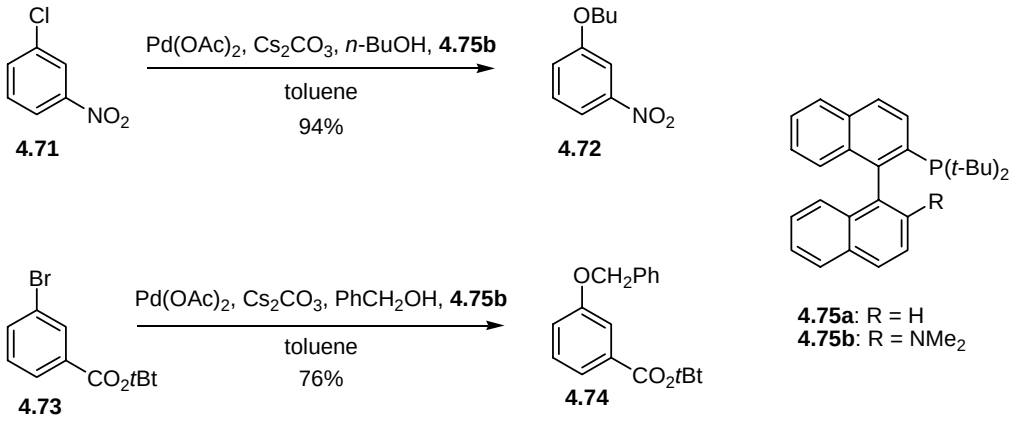
In a similar manner, α-lithiation of the 3-chloro-2-glycosylfuran **4.33** followed by silylation, hydroboration and oxidation delivered β-silanol **4.69**. β-Silanol **4.69** was then converted to **4.70** by reaction with **4.29** under Mitsunobu conditions (Scheme 4.16). Unfortunately the yield was poor (20~30%) despite of many efforts at optimizing the reaction conditions. At this point, we concluded that the activation of the alcoholic hydroxyl as its phosphonium ion would not meet our needs.

Scheme 4.16

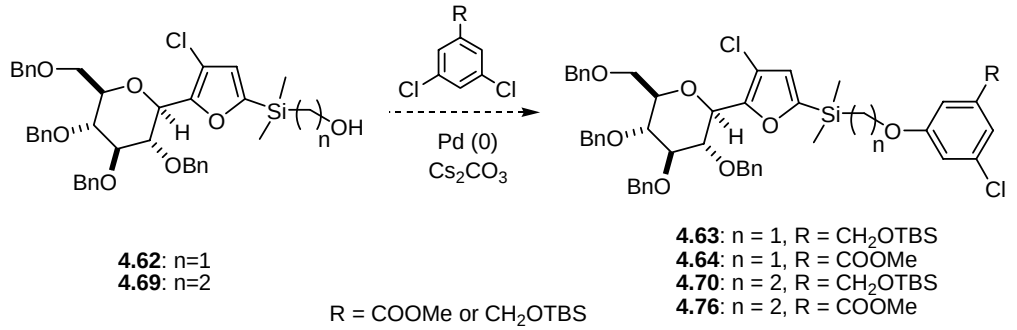


There are other ways to form C-O bonds.[317, 318] For instance, Buchwald recently disclosed a procedure for preparing alkyl aryl ethers *via* a palladium-catalyzed alkylation of an alcohol with an aryl chloride or bromide (Scheme 4.14).[319] By employing the appropriate monodentate biphenyl phosphine ligand **4.75a** or **4.75b**, β-hydride elimination was largely suppressed, and reductive elimination provided the required *C*-O linkage. *m*-Substituted aryl halides with inductively electron withdrawing substituents such as OMe, CO_2Me, CF_3, Ac or NO_2 gave high yields of the alkyl aryl ethers, and functionalities such as ketones, esters, nitriles were well tolerated in the alkylation (Scheme 4.17). Hence a direct transformation to the requisite alkyl aryl ether **4.63**, **4.64**, **4.70** and **4.76** was envisioned as depicted in Scheme 4.18, which, if successful, would afford the cycloaddition precursor in fewer synthetic steps. However, initial attempts with model compounds α-silanol **4.58** and β-silanol **4.66** revealed no formation of the desired alkyl aryl ethers.

Scheme 4.17



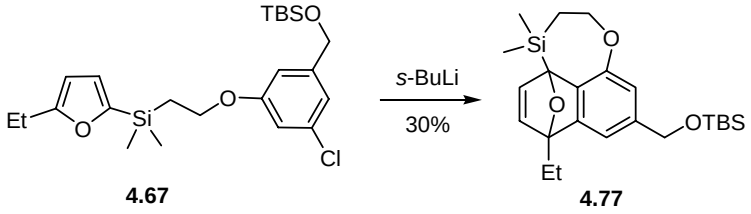
Scheme 4.18



With limited amounts of **4.70** in hand, the key benzyne/furan cycloaddition was explored. It had been found that the model compound **4.67** readily underwent metallation *ortho* to -OMe with excess *s*-BuLi at low temperature. Subsequent warming of the solution afforded the desired cycloadduct **4.77** in an unoptimized yield of 30% (Scheme 4.19). In contrast, metallation on the model **4.59** was accompanied with significant loss of silyl tether probably due to nucleophilic attack of organolithium on the silicon, and no cycloadduct **4.78** was observed (Scheme 4.20). The *ortho*-lithiation of **4.70** was complicated by the loss of the chloride from the furan ring, as evidenced by the upfield shift of the furan proton from 6.7 ppm to 6.4 ppm and a change from a singlet to a

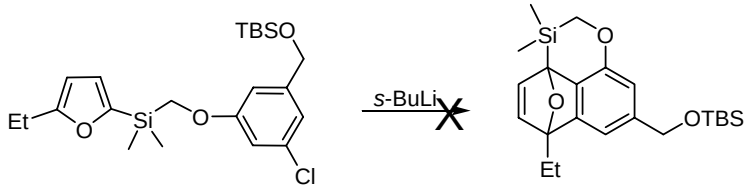
doublet if MeOH instead of MeOH-d_4 was used to quench the reaction. It appeared that lithium-chloride exchange, usually a slow process, took place under the lithiation conditions, probably assisted by one of the many Lewis basic oxygenated functionalities. The unexpected loss of the furan chloride and silyl tether in the *ortho*-lithiation step dealt a final blow to this synthetic route. Because of our inability to effect metallation cleanly and because of the low yields plaguing the etherification reactions, this approach was eventually abandoned.

Scheme 4.19



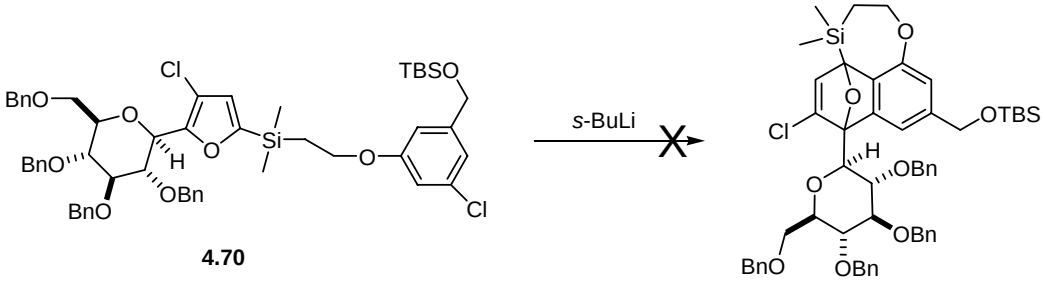
4.67 **4.77**

Scheme 4.20



4.59 **4.78**

Scheme 4.21

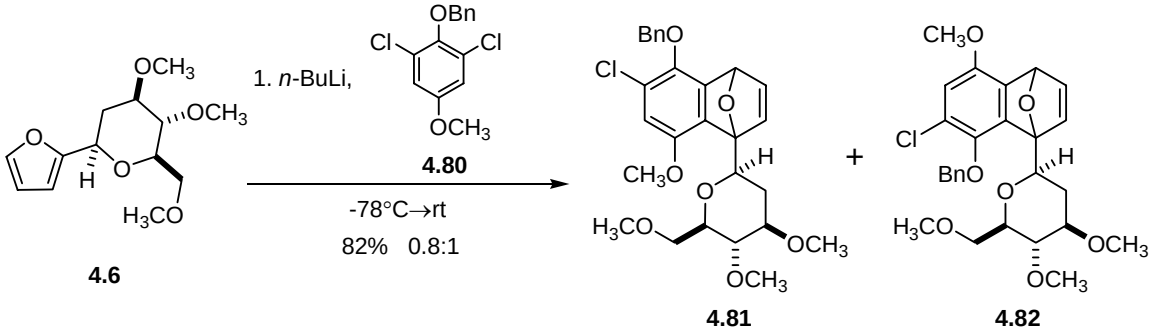


4.70 **4.79**

The Intermolecular Cyclization

In the process of establishing the feasibility of our unified approach to major classes of naturally occurring *C*-aryl glycosides using the glycosyl furan/benzyne cycloaddition, glycosyl-substituted furans possessing no other substituents were universally employed as reaction partners. The frontier orbitals of the dienes are nearly symmetric due to the lack of polarizing groups, and so the global regiocontrol in the cycloadditions had been very poor with ratios of approximately 1:1 commonly encountered. In one of the more selective instances, Dr. Kaelin observed that the cycloaddition of the benzyne derived from **4.80** and 2-glycosyl furan **4.6** gave the expected cycloadducts **4.81** and **4.82** as a mixture of regio- and diastereoisomers (Scheme 4.22). The ratio of **4.81** to **4.82** was determined to be 0.8:1 by integration of the bridgehead protons in the ^{1}H NMR and the structure of **4.82** was assigned by comparison with an authentic sample prepared *via* an alternate approach.[272]

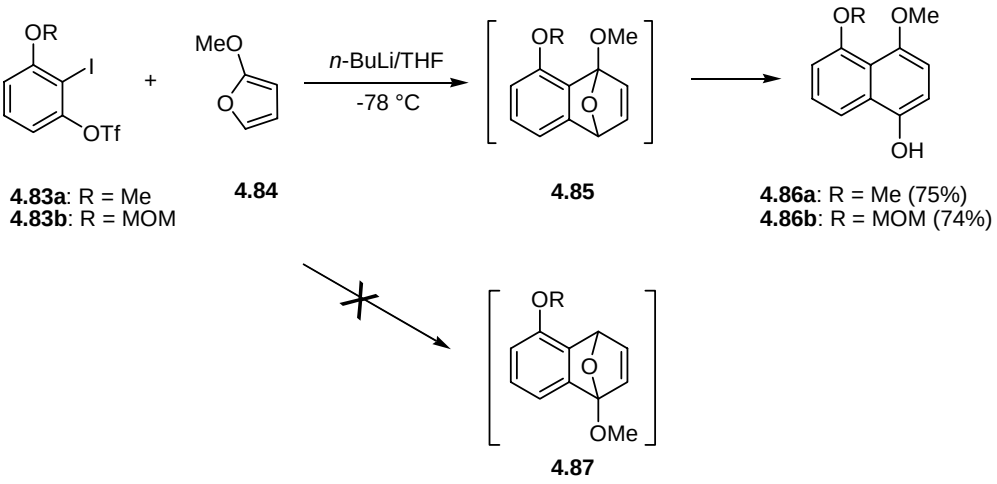
Scheme 4.22



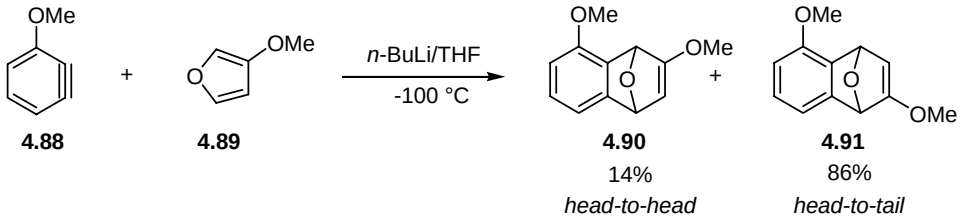
On the other hand, excellent regiocontrol in benzyne/alkoxyfuran cycloadditions were well precedented in literature. For example, Suzuki observed that the head-to-head products **4.86a,b** was dominant in glycosyl-substituted benzyne/2-alkoxyfuran cycloadditions (Scheme 4.23).[172] Sargent observed reversed selectivity in a benzyne/3-

alkoxyfuran cycloaddition, where the head-to-tail product **4.91** was the predominant species (Scheme 4.24).[320, 321] Sargent and Suzuki rationalized the observed regioselectivity based on the polar effect of the alkoxy groups on the dienenophile benzyne and the diene furan

Scheme 4.23



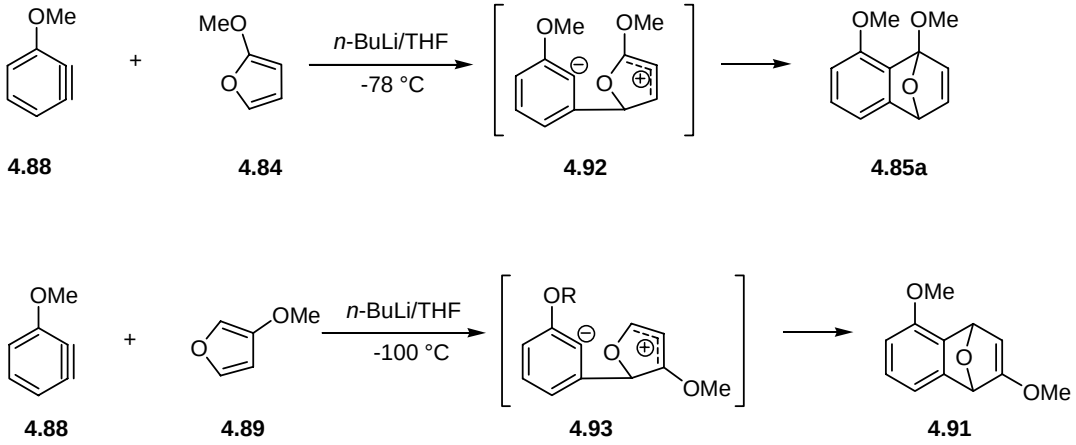
Scheme 4.24



The regioselectivity could be rationalized based on a two-step mechanistic model in which each new bond was formed in a kinetically different step and a discrete zwitterionic intermediate was involved. The zwitterionic intermediate, such as **4.92** and **4.93**, was stabilized by the inductively electron-withdrawing methoxy group on the benzyne and resonance electron-donating methoxy group on the furan. This led to the

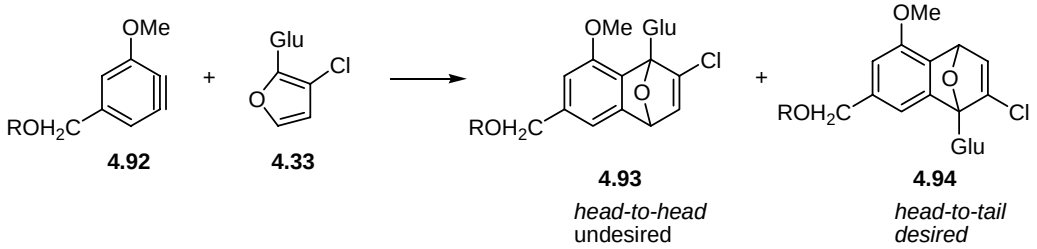
opposite regioselectivity in the cycloadditions involving 2- and 3-alkoxyfurans (Scheme 4.25).[320-322]

Scheme 4.25



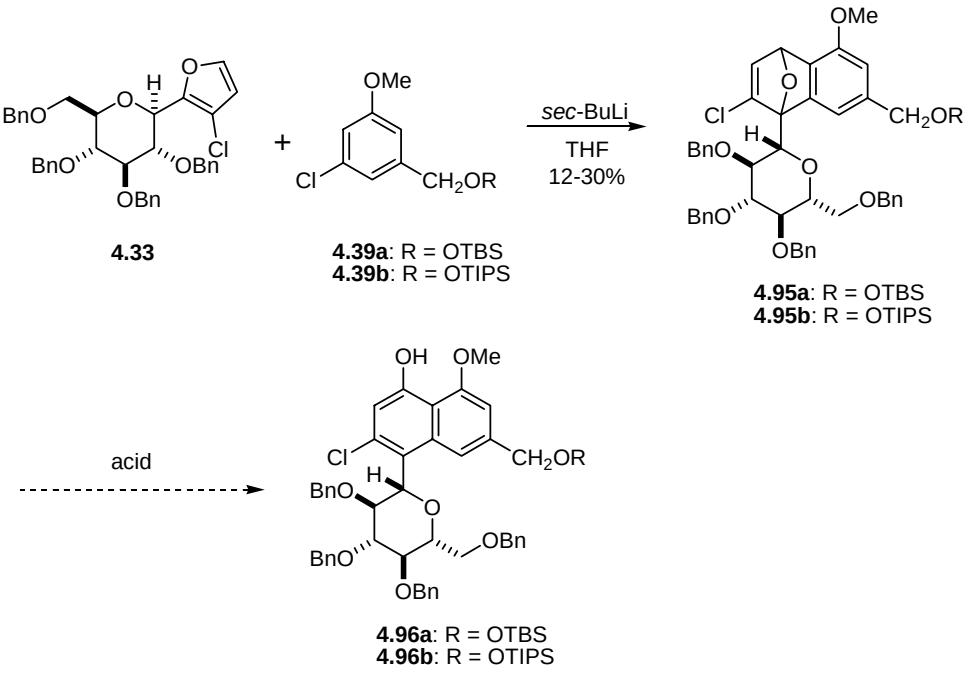
Hence the intermolecular cyclization involving the 3-clorofuran **4.33** was attempted. We reasoned that the inductively electron-withdrawing 3-chloro substituent could potentially polarize the π-system of 3-chlorofuran to induce a regiochemical preference favoring the desired head-to-tail product in the cycloaddition with α-alkoxybenzyne (Scheme 4.25).

Scheme 4.25



The cycloadditions between 3-chloro-2-glycosylfuran **4.33** and benzynes generated from the chloroanisoles **4.39a** and **4.39b** were thus examined. Only one bridgehead proton was observed in the ^{1}H-NMR of the cycloadduct from either reaction (Scheme 4.26). This observation suggested that it was most likely that only one regioisomer was formed, as a large difference in chemical shift of the bridgehead protons between the two regioisomers would be expected. A NOSY experiment confirmed the cycloadducts was **4.95a** was, which was derived from *head-to-tail* cyclization.

Scheme 4.26



4.33

4.39a: R = OTBS
4.39b: R = OTIPS

4.95a: R = OTBS
4.95b: R = OTIPS

4.96a: R = OTBS
4.96b: R = OTIPS

A major concern, however, was that yields were constantly low being no better than 30%, despite efforts to optimize the reaction conditions (Scheme 4.26). A few representative examples of these attempts are presented in Table 4.2. As can be seen, significant amounts of 3-chloro-2-glycosylfuran **4.33** were recovered, even in the presence of large excesses of chloroanisole **4.39a** or **4.39b**. It was later discovered that

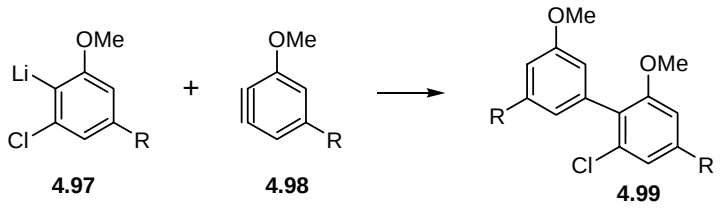
the pathway accounting for the consumption of benzyne was not formation of a cyclobutane *via* benzyne dimerization, but rather the nucleophilic addition of the organolithium **4.97** to the benzyne **4.98** resulting in the formation of a single regioisomer **4.99** (Scheme 4.27), whose structure was identified by ^{1}H-NMR in which no *ortho*-coupling, but only *meta*-coupling, was observed.

Table 4.2: Attempts in the Cycloaddition

entry	reagents			products		
1	solvent	Base	**4.39b**	**4.99**	**4.95b**	Glucofuran **4.33**
2	s-BuLi, THF*	3.0	3.0		26%	
3	s-BuLi, THF	3.0	3.0		26%	55%
4	s-BuLi, ether	3.0	3.0		12%	80%
5	s-BuLi, THF	5.0	5.0	47%	23%	37%
6	s-BuLi, THF	7.5	7.5	52%	20%	37%
7	s-BuLi, THF	10	10	75%	30%	29%

* Glucosyl furan **4.33** was added prior to the addition of *s*-BuLi.

Scheme 4.27



4.97 **4.98** **4.99**

R= CH_2OTIPS or CH_2OTBS

The last and final blow to this approach was the resistance of cycloadducts **4.95a** and **4.95b** to undergo acid catalyzed ring opening to give **4.96a** and **4.96b**, respectively, and we were not able to cleave the oxa-bridge while keeping the rest of the structure intact. When TFA and $BF_3{\cdot}Et_2O$ were used, unchanged starting material was recovered. When more forcing conditions such as TMSOTf was employed, decomposition to a

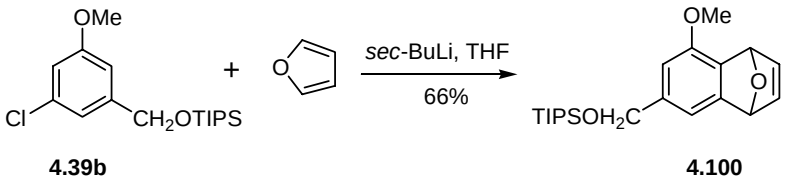
phenol that no longer possesses the carbohydrate moiety became the main reaction pathway. The surprising stability of the cycloadduct toward acids and together with the low yields in the cycloaddition effectively signed the death warrant for this approach.

A Re-examination of the Benzyne/Furan Cycloaddition

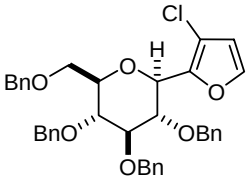
The efforts to control the regiochemistry using a 3-chlorofuran were discontinued, but a question crucial to the success of the project needed to be addressed: What was wrong with the cycloaddition? Dr. Kaelin demonstrated that the cycloaddition between 2-glycosylfuran **4.6** with a 2-deoxy sugar moiety and the symmetric benzyne **4.102** generated from 2-chloro-1,4-dimethoxybenzene (**4.103**) proceeded smoothly to afford the cycloadduct **4.7** in 86% yield (Scheme 4.1). However, we constantly obtained the cycloadduct **4.95** in yields of 20~30% in the cycloaddition of 3-chloro-2-glycosylfuran **4.33** with a non-symmetric benzyne **4.101** (Scheme 4.26). So, what makes our system behave so differently than Dr. Kaelin's? Is it a problem with the glucosyl furan or a problem with the benzyne? A number of studies were initiated to answer those questions.

Benzyne **4.101**, which was generated from chloroanisole **4.39b**, cyclized with furan smoothly (Scheme 4.28), to afford cycloadduct **4.100**, albeit in a lower yield than the reaction with the benzyne from 2-chloro-1,4-dimethoxybenzene (**4.103**) and furan (>80%).[272] The observation that **4.101** participated in benzyne/furan cycloadditions appeared to indicate that the problem in the cycloaddition was not with benzyne **4.101**. Hence the problem seemed to be with the 3-chloro-2-glycosyl furan **4.33**. The major differences between the two glucosyl-substituted furans **4.33** and **4.6** are the presence of an extra chloride on the furan and the replacement of a 2-deoxy sugar with a 2-oxygenated sugar.

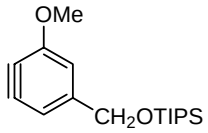
Scheme 4.28



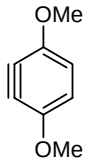
4.39b **4.100**



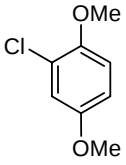
4.33



4.101



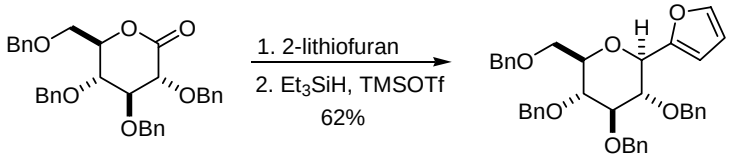
4.102



4.103

MM2 energy minimization using Chem3D suggested that the lowest energy conformer of **4.33** is the one in which the glycosyl substituent is nearly orthogonal to the planar furan ring. Thus the 3-chloro substituent of the furan could have a deleterious effect preventing the rotation of carbohydrate moiety thus blocking the access of the benzyne from either diastereotopic face of the furan. To test this hypothesis, glucosyl furan **4.104** lacking the 3-chloro substituent was prepared using standard procedures as depicted in Scheme 4.29.

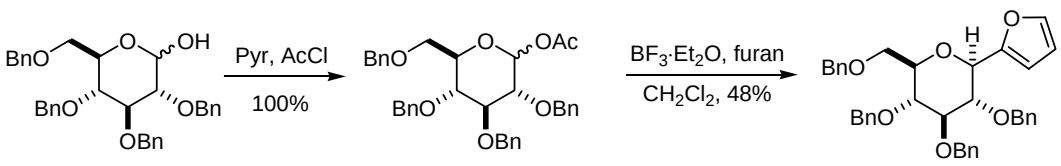
Scheme 4.29



4.31 **4.104**

Alternatively, the 2-glycosyl furan **4.104** was prepared from the anomeric acetate **4.106**, which was allowed to react with furan in the presence of a Lewis acid to afford **4.104** (Scheme 4.30). In both cases, the β-configuration at the anomeric center of the final product was confirmed by comparison with literature reported data ($J_{1,2}$ = 9.7 Hz).[183]

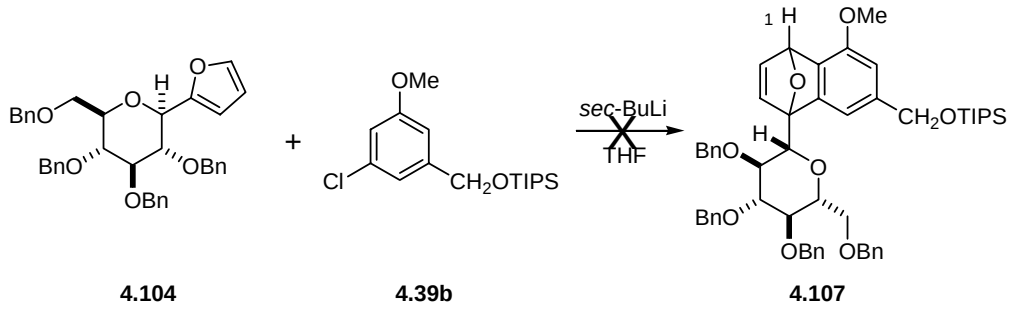
Scheme 4.30



4.105 **4.106** **4.104**

Glycosyl furan **4.104** was then allowed to react with the benzyne **4.101**, which was generated form **4.39b**. However, dimer **4.99** was again the major product, and only traces of the desired cycloadduct **4.107** were observed with the remaining mass being the unreacted glycosyl furan **4.104** (Scheme 4.31). This finding refuted the hypothesis that the presence of the chloro substituent was solely responsible for the low yields in the benzyne/furan cycloadditions of the 3-chloro-2-glycosylfuran **4.33** and the benzyne **4.101**. In general, those cycloadducts were characterized by a distinctive doublet with a smaller coupling constant or a broader single at ~6 ppm in the ^{1}H-NMR corresponding to the bridge-head proton, such as proton 1 in **4.107**.

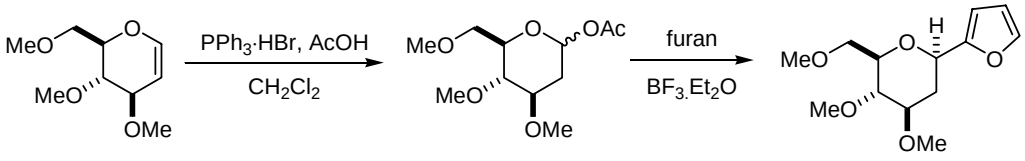
Scheme 4.31



4.104 **4.39b** **4.107**

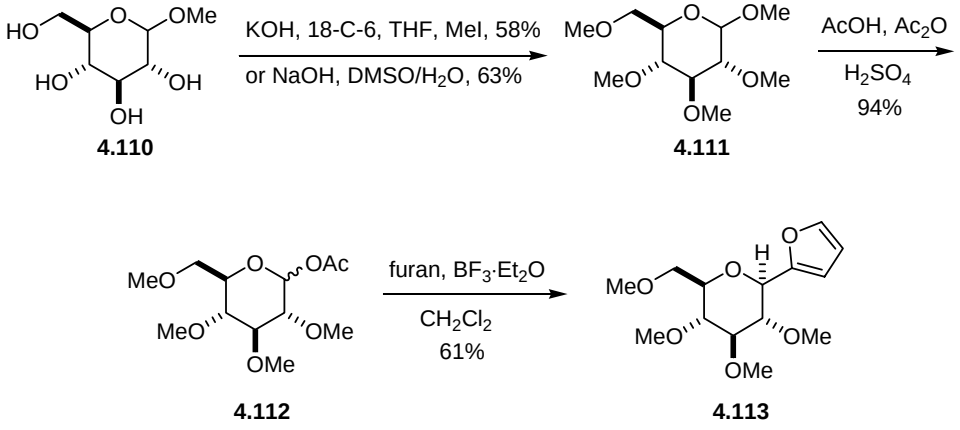
The presence of the oxygenated substituent at the 2-position of the carbohydrate moiety could increase the steric bulk around the reactive center or it could have a negative effect on the conformation of the reactive species, thereby being detrimental to the cycloaddition. To check the validity of this hypothesis, 2-deoxyglucosyl furan **4.6** (Scheme 4.32) and glucosyl furan **4.113** (Scheme 4.33) were prepared *via* Friedel-Craft arylations. The methyl protected compound **4.6** was chosen in preference to the corresponding benzyl protected derivatives to facilitate interpretation of proton NMR. Both Friedel-Craft arylations afforded predominantly the thermodynamically more stable β-anomers under the equilibrating conditions, as evidenced by the large coupling constants ($J_{1,2}$ = 11.7 and 9.7 Hz in **4.6** and **4.113**, respectively) and the occurrence of the anomeric protons at higher field.

Scheme 4.32



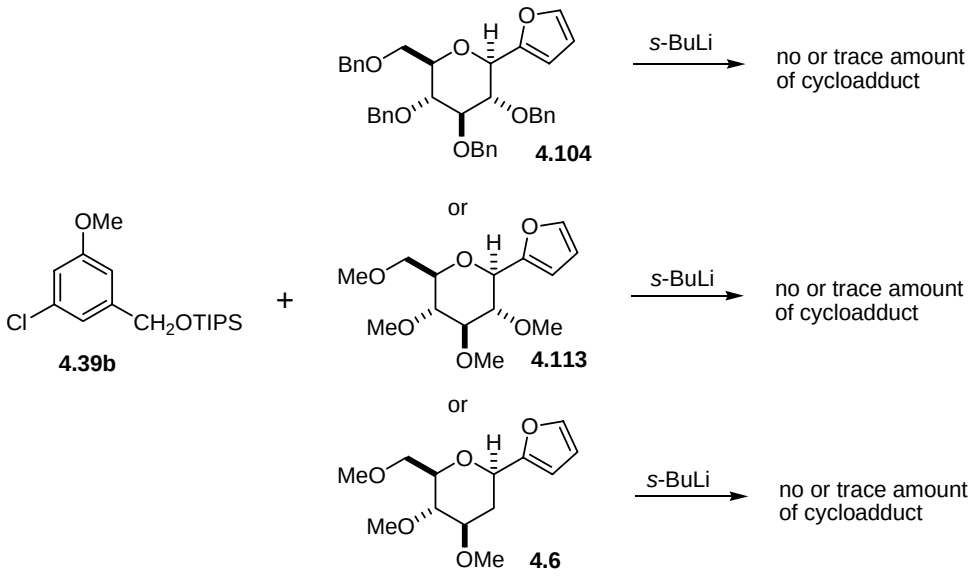
4.108 **4.109** **4.6**

Scheme 4.33



Cycloaddition of the benzyne generated from **4.39b** with either 2-deoxyglucosyl furan **4.6** or the control compound glucosyl furan **4.113** afforded no more than trace amounts of the cycloadducts (Scheme 4.34), which was apparent by examining the ^{1}H-NMR of the crude reaction mixture. This represented a remarkable difference in reactivity, where benzyne generated from **4.103** reacted efficiently with **4.6** (Scheme 4.1). The observation indicated that the 2-oxygenated substituent on the carbohydrate moiety was not the origin for the poor yields in the cycloaddition.

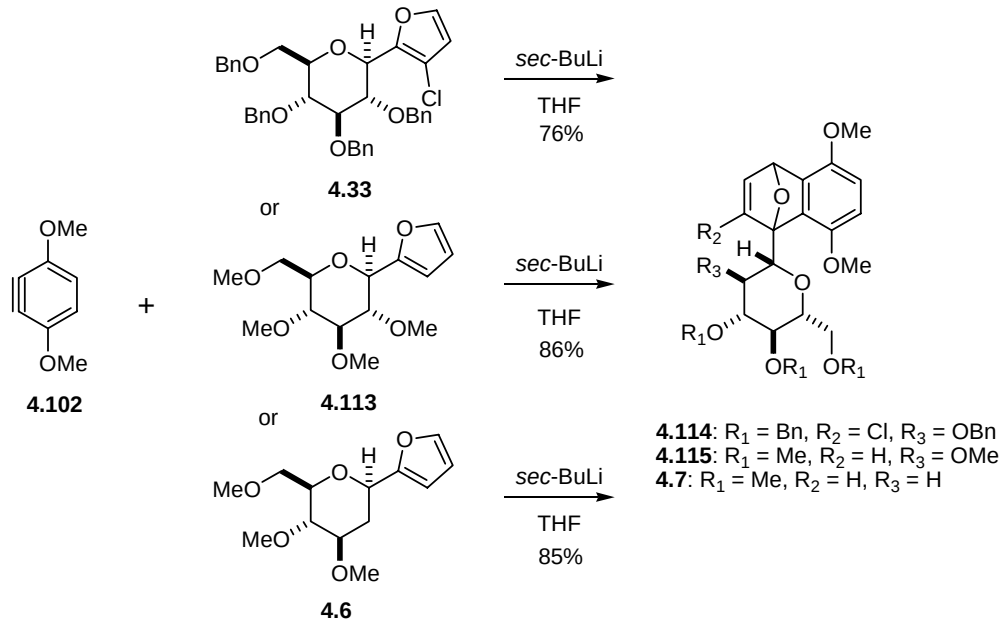
Scheme 4.34



During the course of these studies, it has become clear that not all benzynes are created equal. The high reactivity of benzyne is a doubly edged sword, bestowing the benzyne with the ability to cyclize with the relatively uncreative diene-furan and on the other hand rendering it vulnerable to other side reactions. The observation that benzyne **4.101** does not add efficiently to any of the glycosyl furans thus far tried suggested that it should never be taken for granted that a benzyne, once generated, would cyclize with a substituted furan. Hence an examination of the benzyne **4.101** itself was warranted.

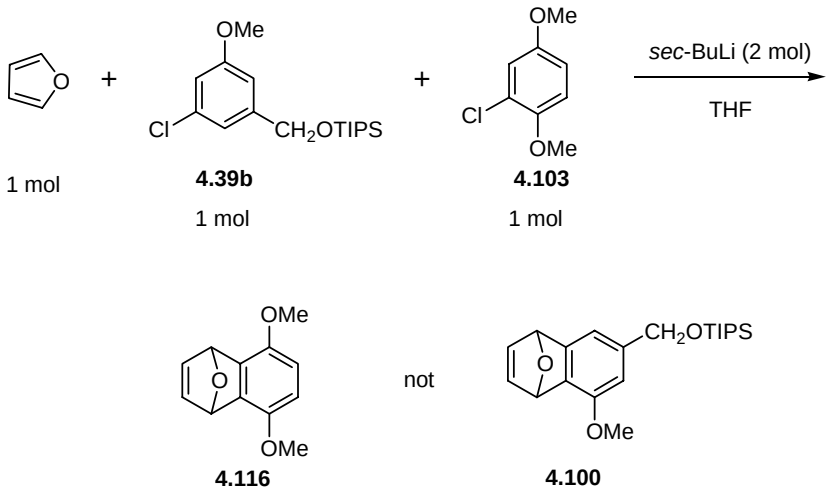
Although the benzyne **4.101** failed to undergo significant cyclization with the three glycosyl furans **4.6**, **4.104** and **4.113** to afford the cycloadducts (Scheme 4.34), the same cyclizations using benzyne **4.102** proceeded smoothly with all three glycosyl furans to afford the desired cycloadducts in good to excellent yields (Scheme 4.35). This observation also reconfirmed the prior conclusion that neither the 3-chloro on the furan nor the 2-oxygenated functionality on the carbohydrate moiety was the primary factor behind the low yields in the cyclizations.

Scheme 4.35



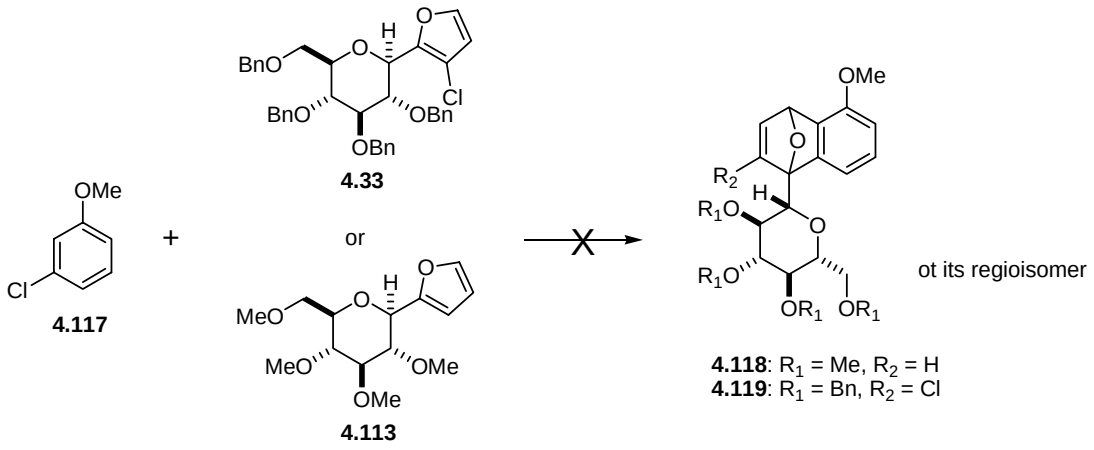
In a competitive trapping experiment, an equimolar mixture of two chloroanisoles **4.39b** and **4.103** was treated with two equivalents of *s*-BuLi at –95 °C in THF. Previously under this condition, benzyne **4.101** and **4.102** had been generated individually and trapped with furan successfully. In the presence of only one equivalent of furan, a single cycloadduct **4.116** rather than a mixture of two cycloadducts **4.116** and **4.100** was observed upon warming (Scheme 4.36). This observation suggested that the benzyne **4.102** might be formed from its corresponding aryllithium at a lower temperature than that required to form benzyne **4.101.** The benzyne **4.102** would then fully consume the furan. Alternatively, the benzyne **4.101** could be a poor dienophile thus failing in the competition with benzyne **4.102** for furan.

Scheme 4.36



The simpler chloroanisoles **4.117** also failed to cyclize with glycosyl furans **4.33** and **4.113** (Scheme 4.37), indicating that the silyloxymethyl group away from the benzyne reactive center was likely not to blame for the low reactivity of the benzyne **4.101** in the benzyne/furan cycloaddition. No control cycloaddition experiment between the benzyne derived from chloroanisoles **4.117** and furan was performed; However, complete deuterium incorporation occurred when **4.117** was treated with *s*-BuLi at –95 ºC followed by quenching with CD_3OD at the same temperature. In analog of **4.39b**, generation of the benzyne from the corresponding organolithium of **4.117** was the likely event during warming up.

Scheme 4.37



Evidently, the second methoxy group on the benzyne had a tremendous effect on its reactivity. The inductively electron-withdrawing methoxy group could lower the LUMO of the benzyne and thus facilitate a normal electron-demand cycloaddition. The presence of the *p*-methoxy group could help stabilizing the zwitterionic intermediate inductively. As a result, the lack of the 4-methoxy functional group in the benzyne **4.101** altered the reaction course, promoting the nucleophilic addition of the organolithium to the benzyne over the cycloaddition with a glycosyl furan and leading to dimer **4.99**.

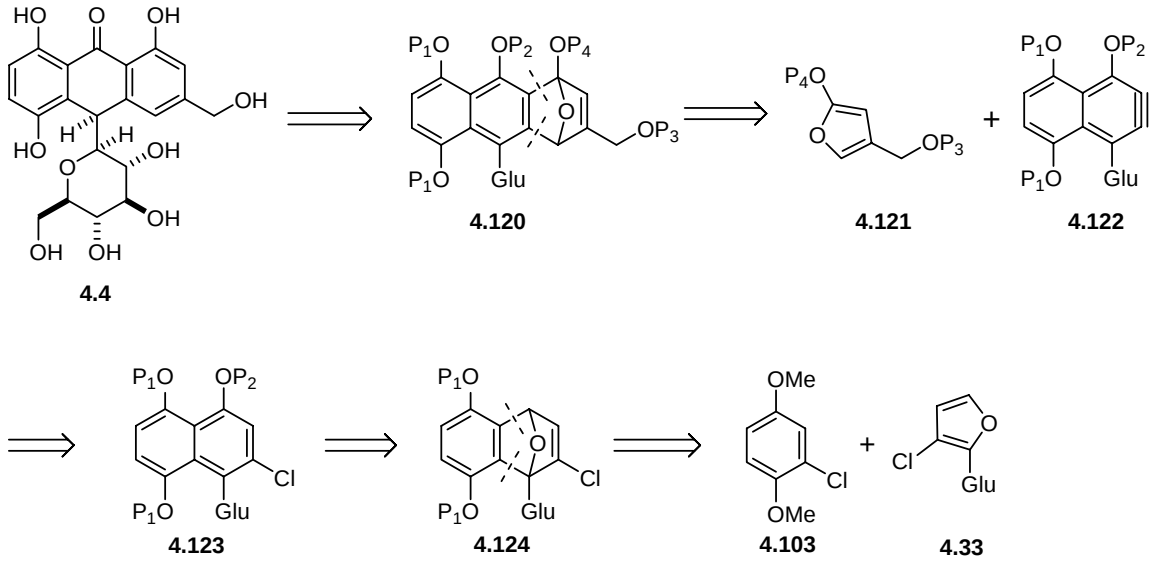
When furan itself was used in the cycloaddition, a satisfactory yield could be obtained with benzyne **4.101** as long as furan was present in large excess (Scheme 4.28). It is not clear whether the same can be said when the structurally more complicated glycosyl substituted furan was employed in excess.

4.2.2 The Second Generation Approach

In view of our discovery regarding the reactivity of benzynes of general type **4.101** and our inability to effect the intramolecular benzyne/furan cycloaddition of **4.70**,

the synthetic approach was revised to preclude the involvement of such a benzyne. The second-generation approach called for initial construction of the AB ring and subsequent appendage of C ring *via* a benzyne/siloxyfuran cycloaddition (Scheme 4.38). This route again featured the same theme of two benzyne/furan cycloadditions. The feasibility of the first of two cycloadditions has already been demonstrated in the earlier section (Scheme 4.35), and the correct regioselectivity in the second cycloaddition between an alkoxyfuran **4.121** and the naphthalene derived benzyne **4.122** was expected based on Suzuki's earlier work in the syntheses of ravidomycin[173, 180] and gilvocarcins.[146] The major disadvantage was the extra phenolic functional group brought in during the second cyclization, which would have to be removed in the subsequent manipulations. The late-stage deoxygenation would present a significant challenge to the synthesis.

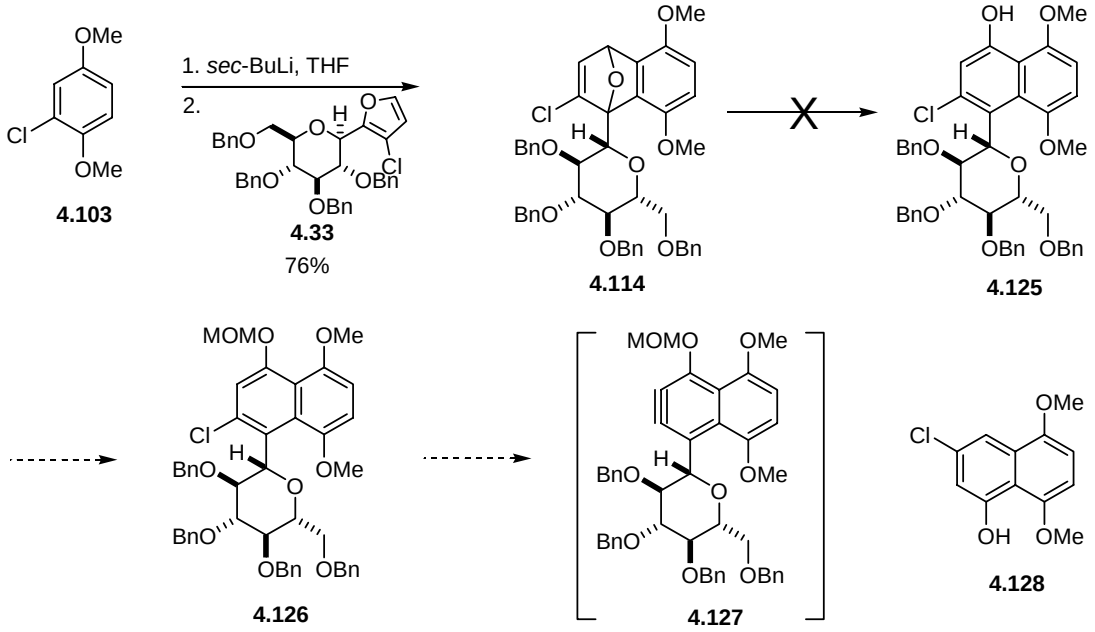
Scheme 4.38



The Use of 3-Chloro Glycosyl Furan

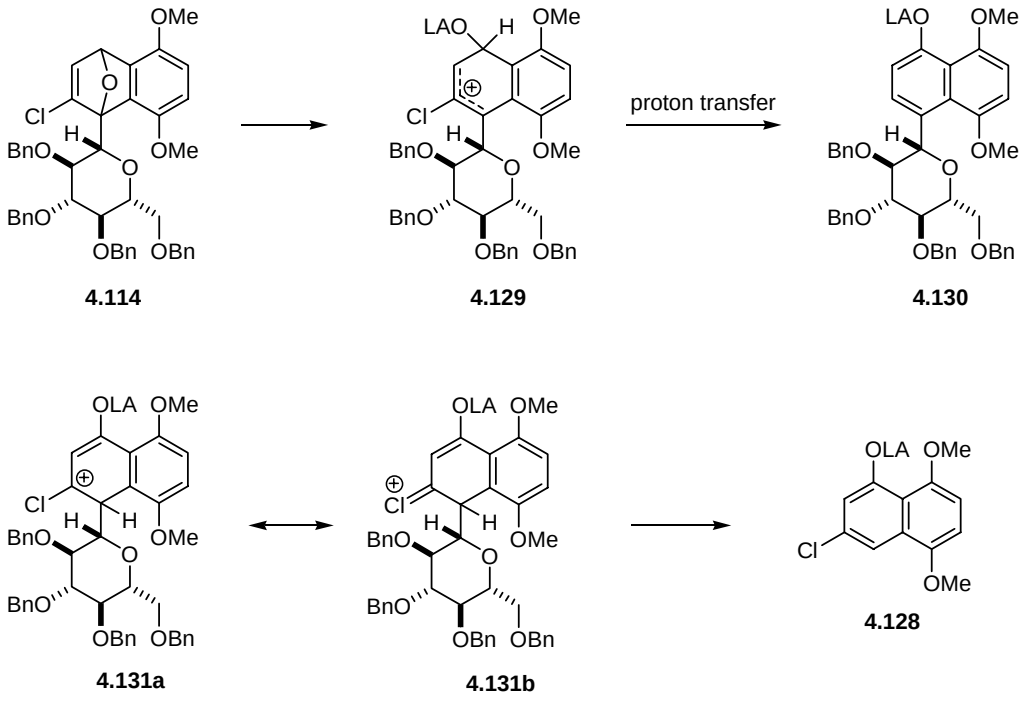
We envisioned that cleavage of the oxa-bridge in cycloadduct **4.114**, which was obtained from cycloaddition of chloroanisole **4.103** and 3-chloro-2-glycosylfuran **4.33**, followed by protection of the naphtholic hydroxyl as its methoxymethyl ether would yield a proper candidate for generation of the second benzyne and set the stage for the final benzyne/furan cyclization. However, the cycloadduct **4.114** persistently defied the acid-catalyzed rearrangement. Under conditions previously used to open said cycloadducts, starting material remained intact even after prolonged periods. At elevated temperatures or in the presence of strong Lewis acids such as TMSOTf, the cycloadduct underwent extensive decomposition with loss of carbohydrate moiety, and instead a naphthol **4.128**, which was identified based on proton NMR, was obtained in 66% yield (Scheme 4.39).

Scheme 4.39



The extraordinary resistance of the cycloadduct **4.114** toward acids could be ascribed to the inductively electron-withdrawing effects of the 3-chloro substituent on the furan and the 2'-benzyloxy group on the carbohydrate moiety. The cooperative effect greatly raised the energy of the intermediate carbocation and consequently severely slowed down the oxabicyclic ring cleavage pathway. The formation of naphthol **4.128** likely proceeded *via* protonation of **4.130** to give **4.131,** which suffered a reversed Friedel-Craft alkylation (Scheme 4.40). Such as mechanism would necessitate the formation of the desired product **4.130** as the intermediate, which was, however, not observed during the reaction.

Scheme 4.40



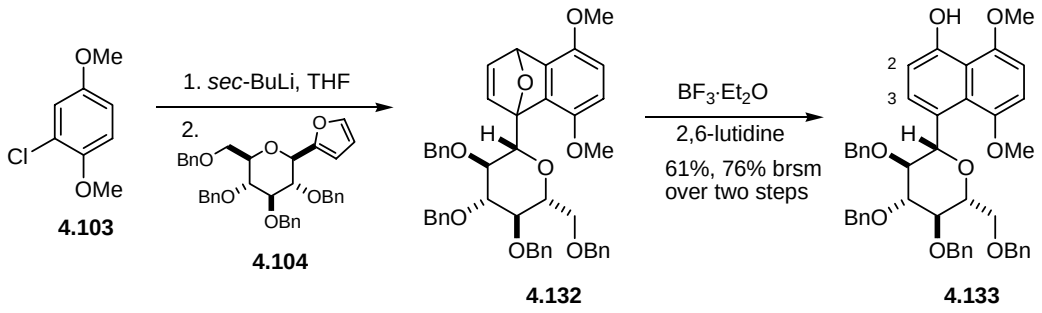
Installation of an electron-donating functionality that would stabilize the developing positive charge in the transition state on either the furan or the carbohydrate

moiety might help. Alternatively one or both of the two electron-withdrawing groups could be removed to increase the stability of intermediate carbocation.

The Use of Glycosyl Furan

Removal of the chloro-substituent from the glycosyl-substituted furan **4.33** would decrease the destabilization of the intermediate carbocation in the acid catalyzed rearrangement. The implementation of such a tactic necessitates installing a chloride on the naphthalene ring at a later stage. Hence, cycloaddition of 2-glycosyl furan **4.104** and chloroanisole **4.103** followed by treatment of the resultant cycloadduct **4.132** with $BF_3{\cdot}Et_2O$ attenuated by 2,6-lutidine afforded the desired naphthol **4.133** in 61% yield over two steps (Scheme 4.41). Use of TMSOTf led to significant decomposition of materials and formation of at least five phenols as shown in the ^{1}H-NMR while $BF_3{\cdot}Et_2O$ alone delivered two phenols in approximate 3:2 ratios.

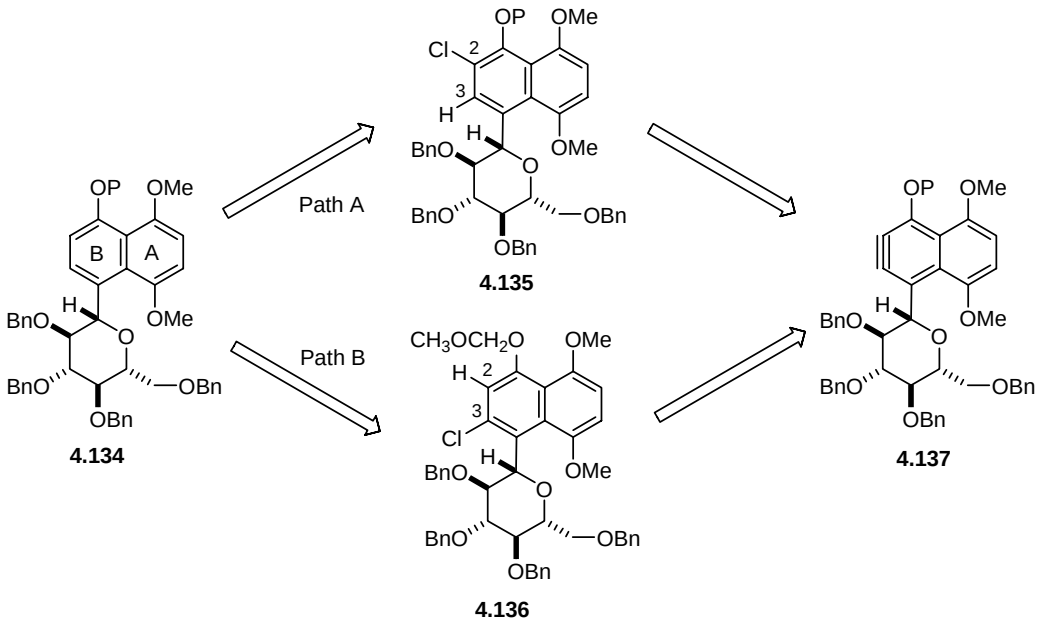
Scheme 4.41



We envisioned a chloride could be installed at either position 2 or 3 of naphthalene B ring of **4.133** to furnish a chloronaphthalene capable of generating the requisite benzyne upon exposure to alkyllithium since both the naphtholic alkoxy and the pyran ring oxygen could serve as the directing group for the lithiation at 2- and 3-

position of the corresponding chloronaphthol **4.136** and **4.135** (Scheme 4.42). Hence, attempts have been made to prepare both the 2- and 3-chloronaphthol **4.135** and.**4.136**.

Scheme 4.42

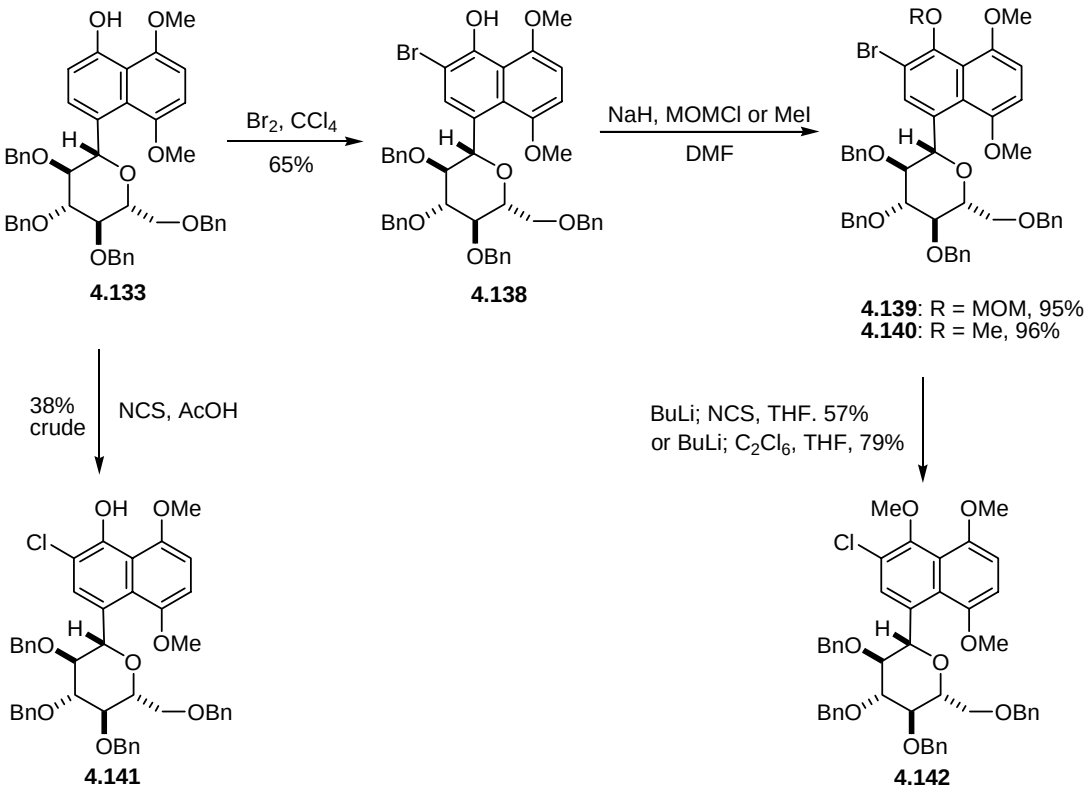


Direct chlorination of the naphthol **4.133** using Sheldon's *ortho*-chlorination protocol with different secondary amines all afforded a variety of unidentified by-products.[323] The desired **4.141** was obtained in moderate yield when NCS was used as the chlorination agent in AcOH; however, it was difficult to purify **4.141** due to the presence of impurities with similar polarity in the reaction mixture (Scheme 4.43).

On the other hand, bromination of naphthol **4.133** using Br_2 in CCl_4 or trimethylbenzyl-ammonium tribromide in CH_2Cl_2/MeOH gave **4.138** in acceptable yield. Hence, the bromonaphthol **4.138** was protected as its methoxymethyl ether **4.139** or methyl ether **4.140**. Compound **4.140** was then converted to its chloro counterpart **4.142**

via lithium-bromide exchange followed by quenching the aryllithium with electrophilic chloride sources, such as C_2Cl_6 or NCS (Scheme 4.43).

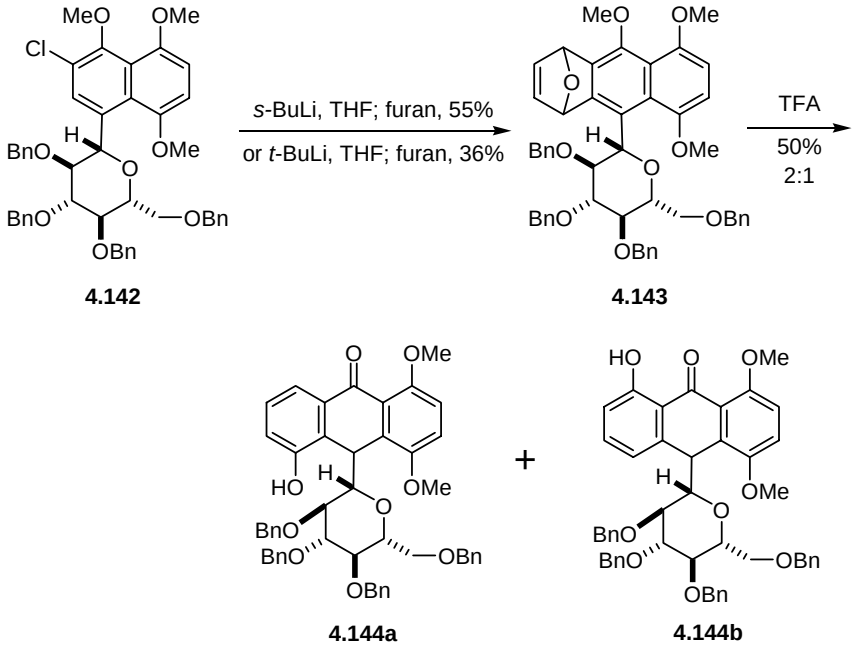
Scheme 4.43



The direct lithiation of the 3-position of bromonaphthol methyl ether **4.140** was first attempted with non-nucleophilic bases.[324] Thus, treatment of **4.140** with LDA at –78 °C followed by quenching with CD_3OD did not result in any deuterium incorporation. When lithium 2,2,6,6-tetramethylpiperidide (LTMP) was used, a complicated mixture was obtained. However, treatment of chloronaphthalene **4.142** with *s*-BuLi or *t*-BuLi followed by quenching with CD_3OD led to significant D-incorporation *ortho* to the pyran ring with no benzylic deprotonation as total integration of benzylic protons remained unchanged. A model benzyl/furan cyclization was attempted in the presence of excess

furan, and we obtained 55% yield of the cycloadduct **4.143**. Exposure of the cycloadduct **4.143** to TFA afforded two compounds, whose structures were assigned to be **4.144a** (33%) and **4.144b** (17%) based on ^{1}H-NMR and MS (Scheme 4.44). Both spectra revealed the loss of an ethereal methyl group. The absence of the anomeric proton from its typical region (6.1 ppm) in ^{1}H-NMR indicated that both compounds existed in their ketone forms. The poor regioselectivity in the opening of the oxabicycle **4.143** was not very surprising in light of Dr. Sparks' observation in the studies towards vineomycinone B2 methyl ester.[278] This lack of selectivity, however, ddidn't present a problem in the natural product synthesis as alkoxyfuran would have to be used in the cycloaddition and the alkoxy group would allow a regiocontrolled ring opening.

Scheme 4.44

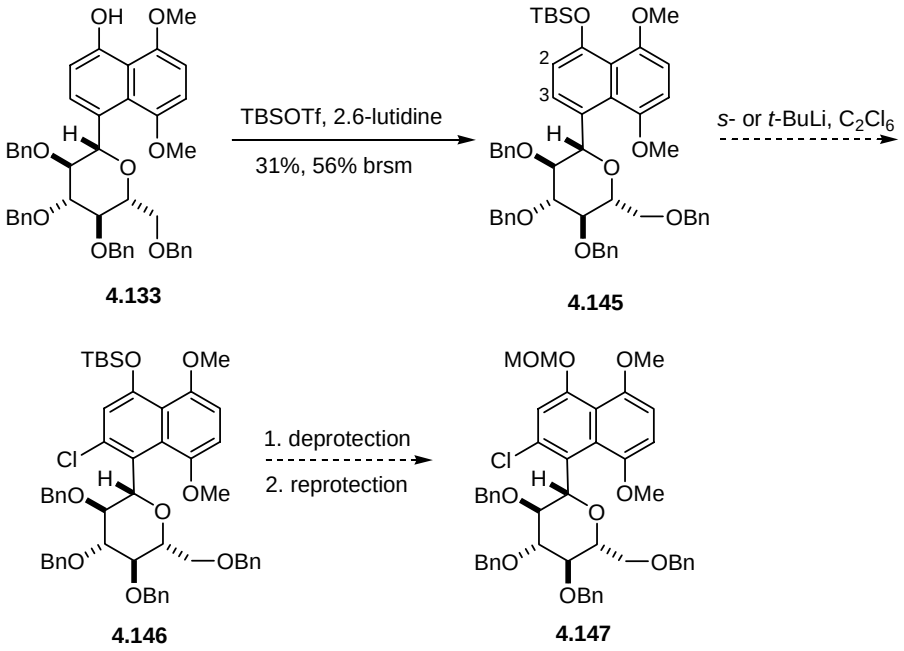


The cycloaddition represented the first successful example where a benzyne was generated from a chloronaphthol precursor **4.142** to form an oxabicylic alkene system.

With proof of concept demonstrated, variation of substitution on the furan partner could afford a cycloadduct that would lead to 5-hydroxyaloin A.

A 3-chloronaphthol such as **4.125** could have been available if the benzyne/furan cycloaddition with 3-chloro-2-glycosylfuran **4.33** and subsequent acid-catalyzed ring opening had been successful (Scheme 4.39). Alternatively, 3-chloronaphthol such as **4.125** could be accessed by metallating the 3-position of a protected naphthol of form **4.134**, which would be directed by the pyran ring oxygen, followed by electrophilic chlorination. Toward this goal, the naphthol **4.133** was treated with TBSOTf and 2,6-lutidine to give **4.145** in moderate yield (Scheme 4.45). Use of TBSCl/imidazole in DMF afforded a mixture (1:1) of the desired product **4.145** and an unidentified side product together with significant amount of unreacted starting material, even after prolonged stirring. The lack of reactivity reflected the generally low reactivity of a hydrogen bonded aromatic hydroxyl functional group and probably also the relative steric hindrance around the reacting center in naphthol **4.133**. The presence of an intramolecular hydrogen bond was well documented and has been known to be the cause of the downfield shift of the naphtholic hydroxyl group in 8-methoxy-1-naphthol systems. However, treatment of **4.145** with butyllithium or LDA followed by quenching the mixture with CD_3OD reveled no deuterium incorporation and **4.145** remained intact in all the cases, hence the effort to prepare the benzyne precursor **4.147** was discontinued.

Scheme 4.45

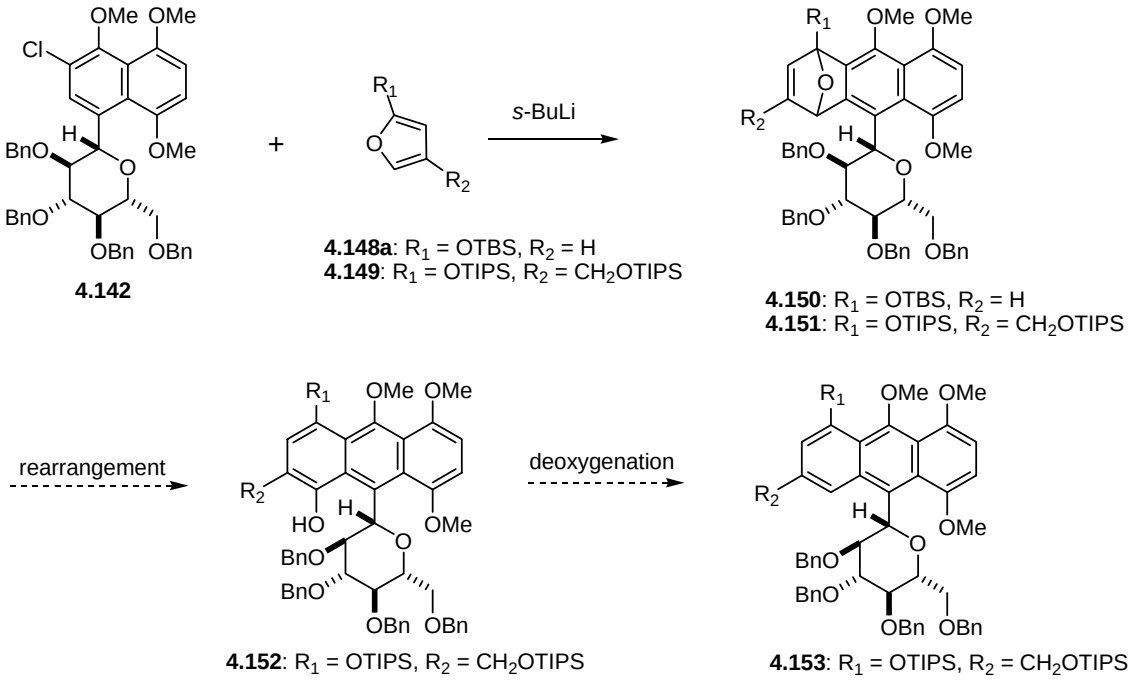


The observation that the pyran ring oxygen was not potent enough to promote the *ortho*-lithiation of **4.145** raised a question as to whether the same oxygenated functionality could act as the directing group in the *ortho*-lithiation of **4.142**. Iwao reported that the direct lithiation of chlorobenzene with *s*-BuLi at –105 °C in THF, suggesting chloride alone might be enough to induce *ortho*-deprotonation.[325]

Cycloadduct **4.143** lacks the proper functionalities to be elaborated easily into the 5-hydroxyaloin A (**4.4**). To access the natural product efficiently, it was desirable to have the requisite functionality already present on the cycloadduct, this requirement necessitated the use of a siloxylfuran such as **4.149** as the dienic partner. It would then be necessary to remove the anthracenol hydroxyl from **4.152** (Scheme 4.46). The corresponding cycloaddition of **4.142** and siloxyfuran **4.148a** or **4.149** appeared to have produced the desired cycloadducts **4.150** and **4.151** as indicated by the ^{1}H NMR of the

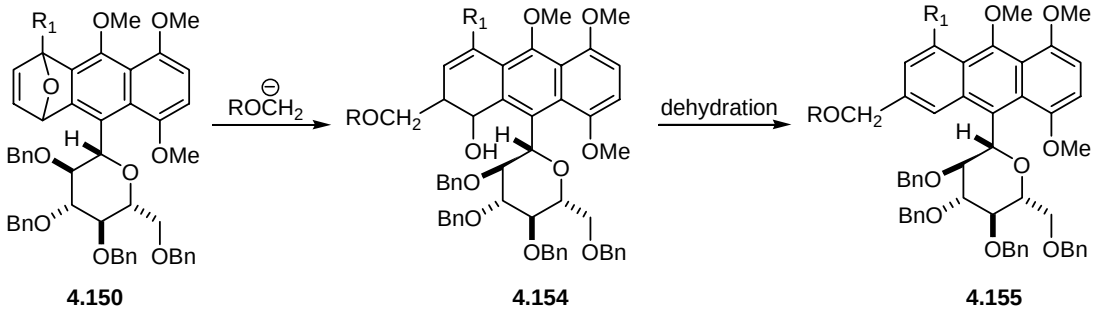
crude material and LRMS. However, a number of unidentified impurities were inevitably produced in the cycloaddition. Because the cycloadduct was not particularly stable, it was never successfully purified to homogeneity. On the other hand, even if the cycloadduct **4.151** could be purified and converted to anthracenol **4.152** cleanly, the deoxygenation at a late stage of the synthesis would likely be problematic.

Scheme 4.46



To address this problem, a slightly different strategy was developed involving the S_N2' ring opening of the cycloadduct **4.150** with a hydroxymethyl anion equivalent followed by dehydration to eliminate the extra hydroxyl and aromatize the system (Scheme 4.47). Such dehydrations were known to be facile transformations and often occurred spontaneously without the activation of alcohol.[269, 270]

Scheme 4.47

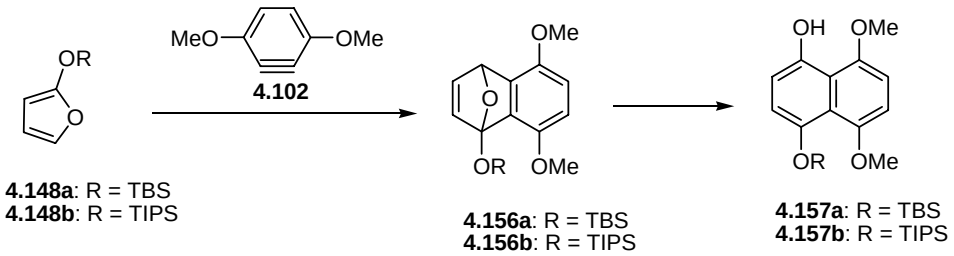


4.150 **4.154** **4.155**

The Deoxygenation via Dehydration

A model system was designed to explore the feasibility of this strategy. The oxabicyclic alkenes **4.156a,b** were prepared from the benzyne/siloxyfuran cyclization of **4.102** and silyloxyfurans **4.148a,b** (Scheme 4.48). Because these compounds were unstable, they were purified by partitioning between acetonitrile and hexanes. Rapid decomposition of **4.156a,b** to naphthols **4.157a,b** was observed on contact with chromatographic medias such as silica, Et_3N attenuated silica, basic alumina and neutral alumina. The purified cycloadducts **4.156a,b** as pale yellow solids slowly darkened to orange even when stored in freezer and under argon. This extremely facile ring opening posed a serious problem to the proposed S_N2' ring opening/dehydration sequence.

Scheme 4.48



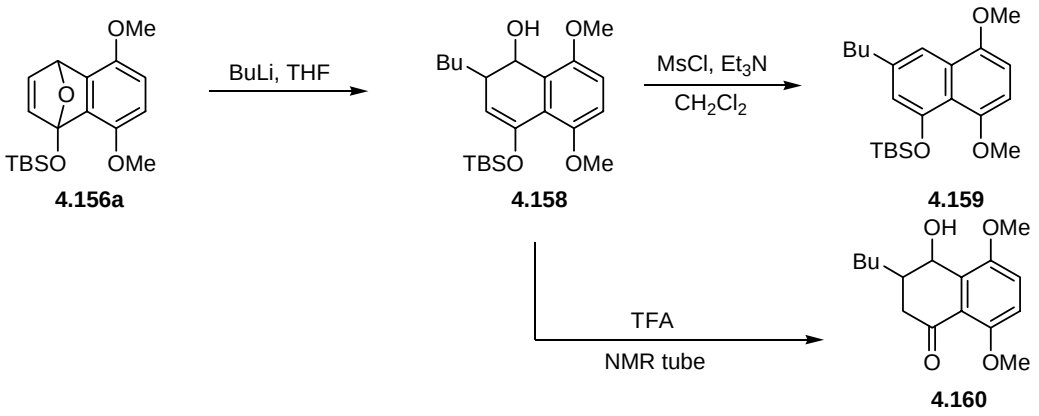
4.148a: R = TBS
4.148b: R = TIPS

4.156a: R = TBS
4.156b: R = TIPS

4.157a: R = TBS
4.157b: R = TIPS

Initial attempts indicated that the oxabicycle **4.156a** could be opened with *n*-BuLi to give **4.158** (Scheme 4.49). However, various amounts of naphthol **4.157a** were inevitably formed in all the cases, presumably because of the Lewis acidity of the lithium cation. Addition of Lewis acid such as $BF_3{\cdot}Et_2O$ greatly promoted the formation of naphthol **4.157a**. Dehydration of the product mixture above obtained with MsCl in the presence of Et_3N afforded the desired naphthalene **4.159** smoothly. Attempted dehydration of **4.158** with TFA, however, afforded a different product, which was tentatively assigned as the ketone **4.160** based on ^{1}H-NMR and IR.

Scheme 4.49

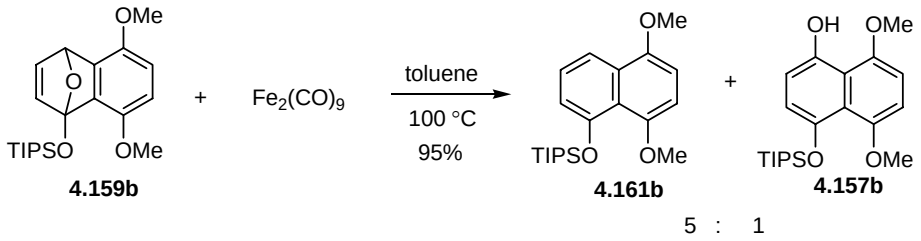


Reaction of **4.156a,b** with the less nucleophilic MeLi, however, was unsuccessful. Methyllithium alone failed to open the cycloadducts **4.156a,b** with or without activation of a weak Lewis acid; naphthols **4.157a,b** were the only products observed in the reactions. Even under strongly nucleophlic conditions, as in the presence of HMPA, 12-*C*-4, DMPU or TMEDA, opening of the oxa-bridge to form naphthols dominated the reactions, and no trace of the desired product was observed in ^{1}H-NMR. Attempted coupling of **4.156b** with organocuprates, generated from corresponding Gringard reagents, gave only the naphthol **4.157b** together with recovered starting material. The

S_N2' displacement with methoxymethoxymethylithium, which was generated from methoxymethoxymethyltributylstannane[326] and BuLi, was attempted, but no desired product was observed. This approach was hence abandoned since the desired S_N2' ring opening of the cycloadducts **4.156a,b** didn't compete favorably with the facile ring-opening process.

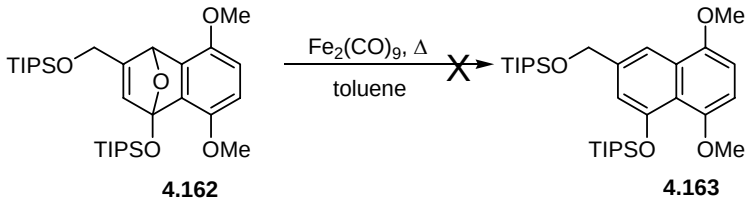
Wege discovered that 1,4-epoxy-1,4-dihydroarenes can be converted in high yields into the corresponding arenes with nonacarbonyldiiron in refluxing benzene. Indeed, heating a mixture of **4.156b** and $Fe_2(CO)_9$ afforded the desired product **4.161b** in good yield (Scheme 4.50), although small amount of the corresponding naphthol **4.157b** was also formed.

Scheme 4.50



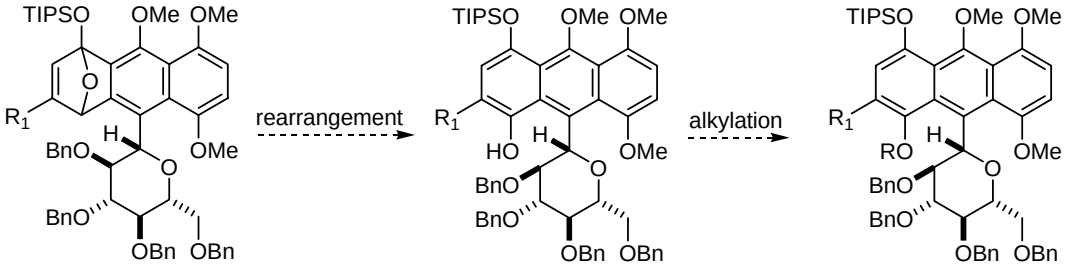
To apply this methodology to our synthesis, a hydroxymethyl substituted siloxyl furan was required in the benzyne/furan cycloaddition. The model oxabicycle **4.162** was thus prepared, but it was fairly unstable. When the crude **4.162** was subjected to Wege's conditions, the desired arene **4.163** was not formed (Scheme 4.51).

Scheme 4.51



Having failed to effect the nucleophilic opening and direct reductive aromatization of the oxabicycle, we were forced to go through the route involving deoxygenation of the anthracenol **4.152**. However, the corresponding cycloadduct **4.151** was very unstable. Attempted opening of **4.150** and **4.151** all produced mixtures of compounds that offered little if anything conclusive. The anthracenol **4.152** may have been formed as a phenolic peak was observed in the ^{1}H-NMR. Attempts were also made to protect the anthracenol hydroxyl group of **4.152** as it acetate or alkyl ether **4.164** *in situ* in hope that a stable compound could be isolated (Scheme 4.52). However, mixtures of unidentified compounds were always obtained, and it was not clear whether the anthracenol **4.152** was actually formed in the first step of the reaction. Due to the unavailability of the anthracenol **4.152** at this stage, the deoxygenation hence cannot be explored.

Scheme 4.52



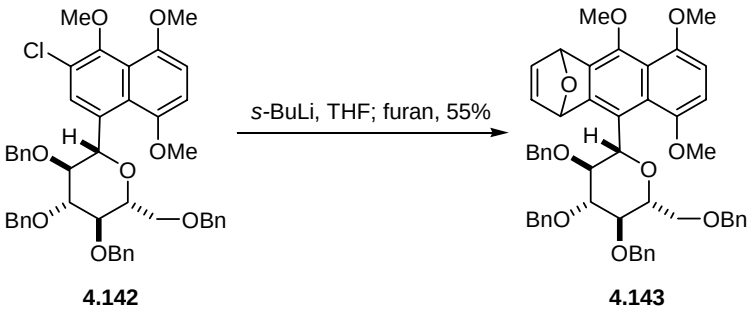
4.151: $R_1 = CH_2OTIPS$ **4.152**: $R_1 = CH_2OTIPS$ **4.164**: $R_1 = CH_2OTIPS$

If the deoxygenation was successful, the rest of the synthesis only entails deprotection of all the protecting groups. Spontaneous enol-ketone tautomerization would then afford 5-hydroxyaloin A.[327]

To emphasize, proof of concept was established in our two-stage benzyne/furan cycloaddition strategy to assemble the anthrone core of the 5-hydroxyaloin A. A benzyne

can be generated from a chloronaphthol precursor **4.142** and subsequent cycloaddition with furan afforded the cycloadduct **4.143** (Scheme 4.53).

Scheme 4.53



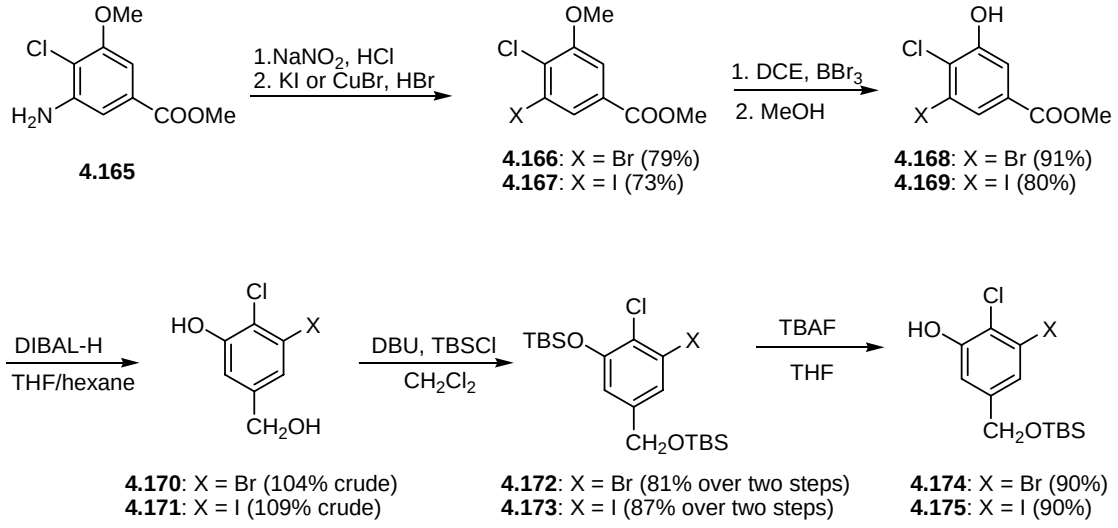
4.142 **4.143**

4.2.3 Revisiting of the Tether Strategy

It was an obvious disadvantage that the unsymmetrical C ring had to be constructed at the final stage of the synthesis with the proper functional groups attached. Strategically, it was better to form the C ring at the onset of the synthesis. Hence the tether approach was revisited and revised, and we decided this time to use a dihalopehnol as the benzyne precursor to address two primary issues in the prior synthesis: These are the low yield in the etherification and the loss of the furyl chloride during lithiation. The lower pKa of the dihalophenol could facilitate the etherification and help improving the chemical yield. The facile lithium-bromide exchange can thwart undesired lithium–chloride exchange previously observed during the lithation of **4.70**. The major issue at stake was the reactivity of the tether benzyne, which had been known to be a poor dienophile when not tethered. Tethering the benzyne and furan partner together would significantly increase the effective concentration of the glycosyl furan and thereby potentially compensate the low reactivity of the benzyne.

The synthesis of dihalo phenols **4.174** and **4.175** started with the known aniline **4.165** (Scheme 4.54).[328, 329] The free amino group of **4.165** was transformed into a bromide or an iodide through its diazonium salt under Sandmeyer conditions, and the aromatic methyl ether was selectively deprotected in the presence of the methyl ester. However, it is operationally more convenient to effect global deprotection and then reform the methyl ester. The methyl ester **4.168** and **4.169** were then converted to phenols **4.174** and **4.175** under standard conditions.

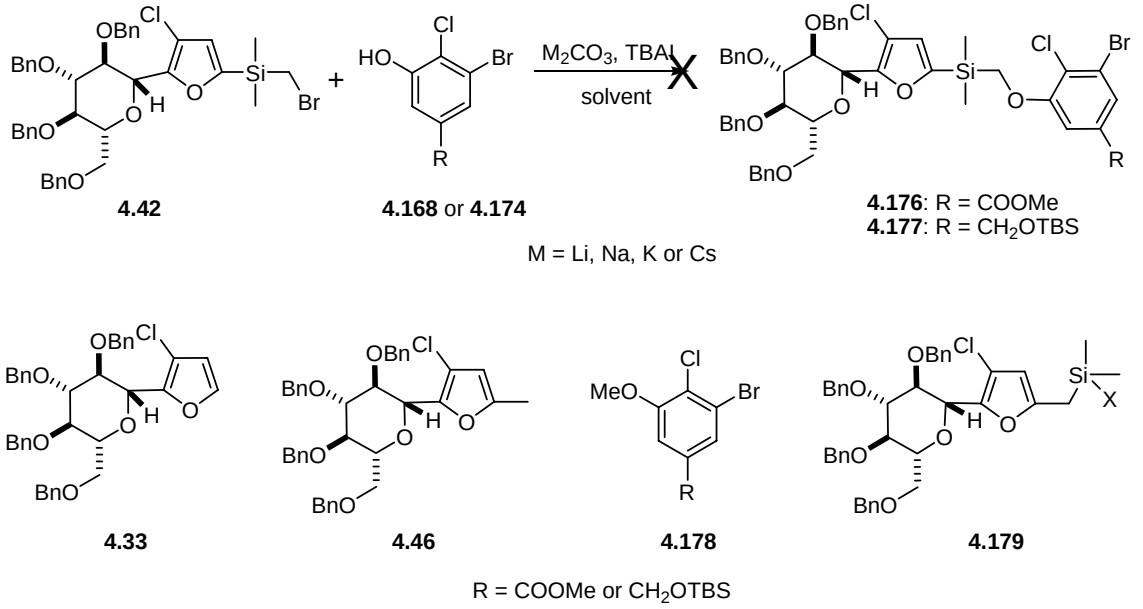
Scheme 4.54



The etherification of α-bromosilane **4.42** with phenols **4.168** or **4.174** was again troubled with the infamous α-halosilane rearrangement. The desired product was barely formed, and instead by-products **4.33, 4.46, 4.178** and **4.179** were isolated in various ratios dependent on the reaction conditions (Scheme 4.55). The presence of the anisole **4.178** in the reaction mixture appeared to suggest that the desired product **4.176** or **4.177** may have formed during the course of the reaction. Subsequent desilylation of **4.176** or

4.177, either by the action of an acid or a nucleophile would provide methylated phenol **4.178**.

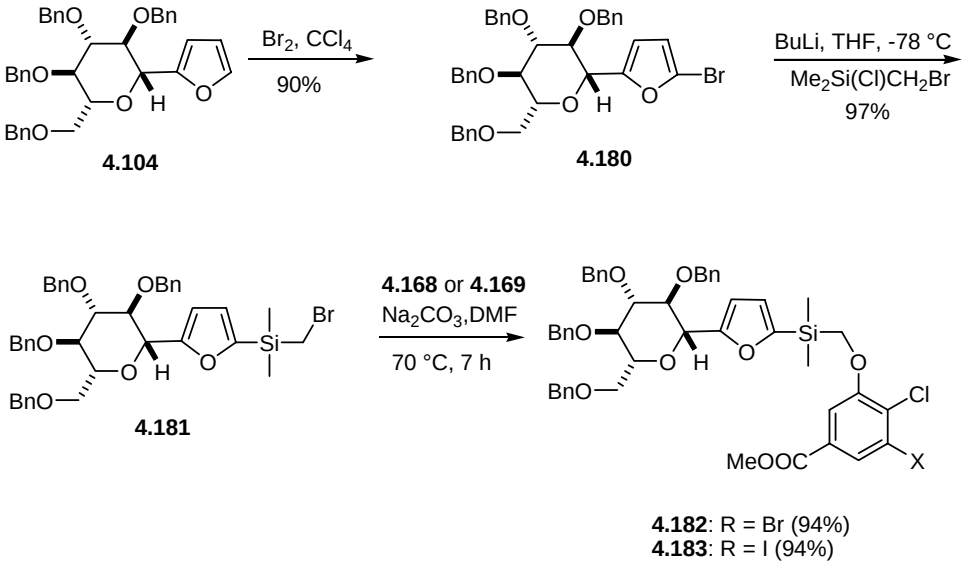
Scheme 4.55



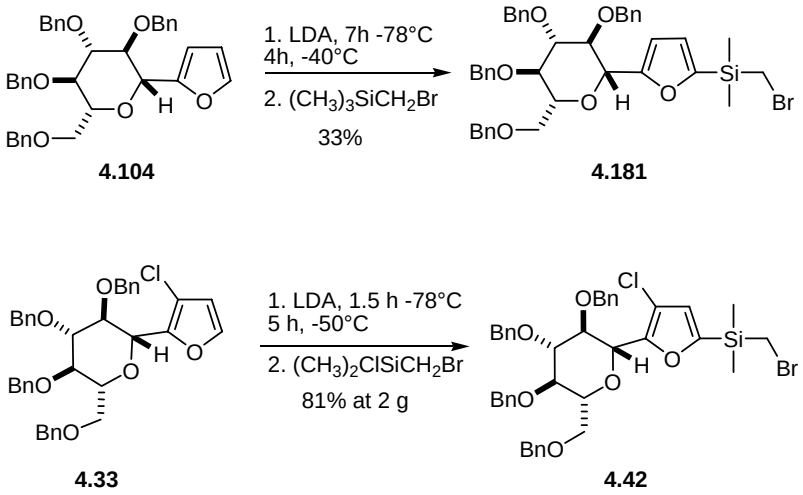
Etherification of the phenols **4.168** and **4.169** with bromomethylsilane **4.181** lacking the furyl chloride substituent, however, proceeded smoothly to afford the desired products **4.182** and **4.183** respectively in excellent yields when the reactions were run in DMF in the presence of sodium carbonate as base (Scheme 4.56). The reaction was highly sensitive to the nature of the metal counter ion of the carbonate base and the solvent, as the potassium and cesium carbonate caused extensive α-halosilane rearrangement and the rate of the etherification was significantly slower in acetonitrile and acetone. The bromosilane **4.181** was prepared *via* the bromofuran **4.180**, which was converted to the corresponding organolithium upon treatment with *n*-BuLi. The direct lithiation of **4.104** followed by silylation with either bromomethylchlorodimethylsilane or

chlorodimethylvinylsilane gave only poor yields of the desired silanes, and significant amounts of the starting material remained even after prolonged period of lithiation (Scheme 4.57). This presented another sharp difference in reactivity between the 2-glycosyl furan **4.104** and the 3-chloro-2-glycosylfuran **4.33**.

Scheme 4.56

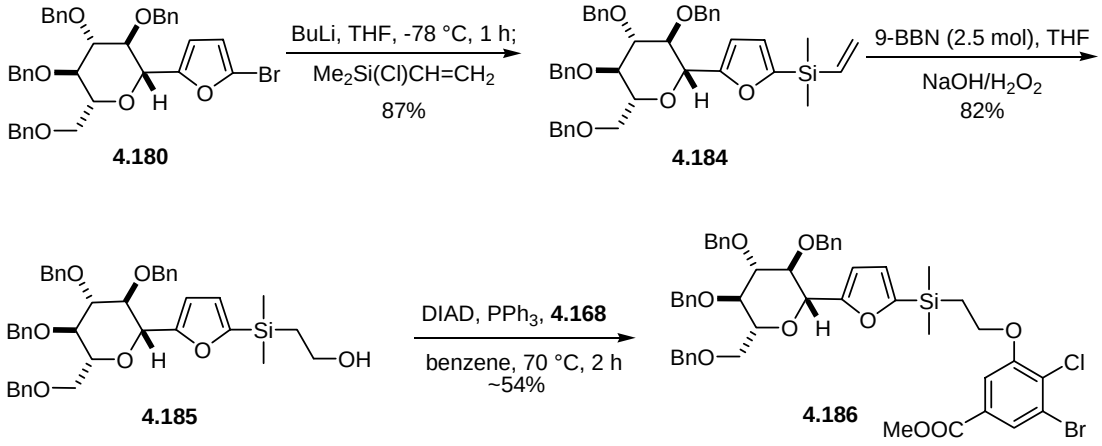


Scheme 4.57

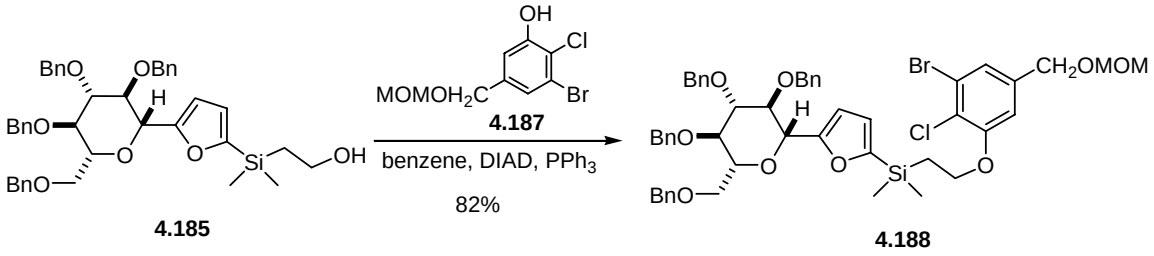


In a similar manner, the vinyl silane **4.184** was prepared from 2-bromofuran **4.180**. Metal-halide exchange followed by quenching the resultant furyllithium with silyl chloride provided vinyl silane **4.184** in excellent yield. Compounds **4.184** was then converted into the β-silanol **4.185** by hydroboration with 9-BBN and oxidation with basic peroxide. Mitsunobu etherification of the β-silanol **4.185** and the phenol **4.168** afforded the desired product **4.186** in acceptable yield (Scheme 4.58). Significant improvement in yield was observed when the benzyl hydroxyl protective group was switched from a TIPS silyl ether to a methoxymethyl ether (Scheme 4.59).

Scheme 4.58

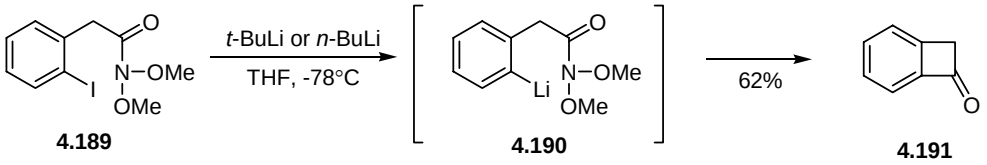


Scheme 4.59



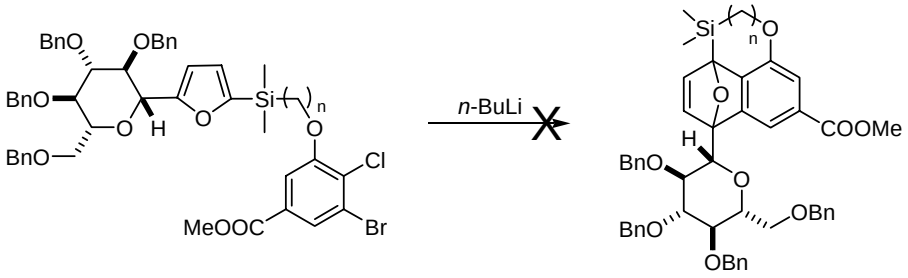
The success in the etherification set the stage for the key cyclization. The lithium-bromide exchange, which was used to generate the requisite aryllitihum, is a facile process.[330, 331] It has been reported that the rate of lithium-halogen exchange can exceed the rate of proton transfer in some instances,[331] and the exchange is typically more rapid than addition reactions that might compete (Scheme 4.60).[330] Hence the intramolecular cycloaddition of **4.182** and **4.186** was attempted at room temperature in hope that the lithium-halide exchange and subsequent elimination of the chloride and cyclization could effectively compete against the attack on the methyl ester by the organolithium. However, when a THF or ether solution of **4.182** or **4.186** was treated with *n*-BuLi at –78, 0°C or rt, only intractable mixture consisting of many unidentified compounds was obtained in each case (Scheme 4.61).

Scheme 4.60



4.189 **4.190** **4.191**

Scheme 4.61

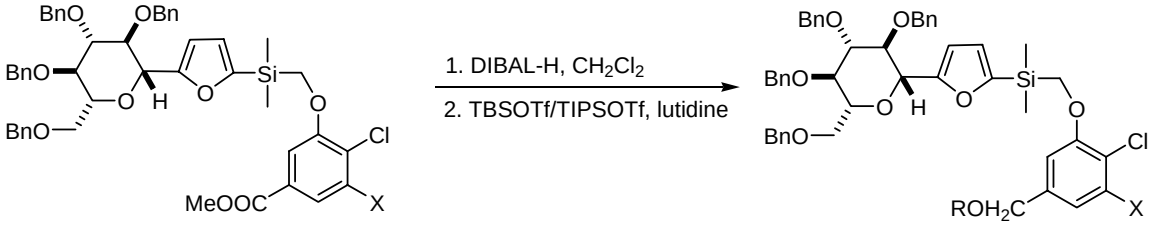


4.182: n =1
4.186: n =2

4.192: n =1
4.193: n =2

The methyl esters **4.182**, **4.183** and **4.186** were thus reduced to the benzylic alcohols, which were then protected as their silyl ethers **4.194**, **4.195** and **4.196**, respectively (Scheme 4.62 and Scheme 4.63). Alternatively, the requisite ethers, such as **4.197** can be prepared directly *via* Williamson etherification of the bromide **4.181** and an appropriate phenol in moderate yield (Scheme 4.64).

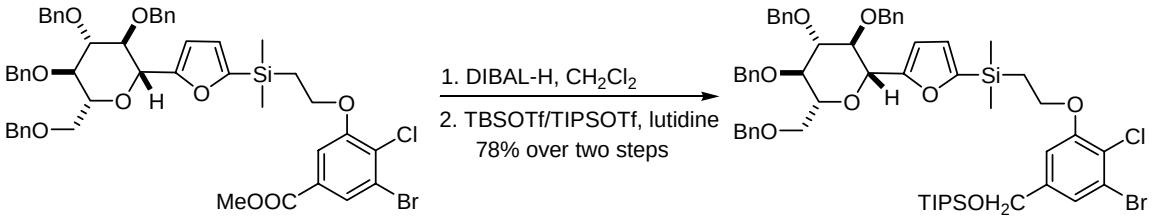
Scheme 4.62



4.182: R = Br
4.183: R = I

4.194: X = Br, R = TBS (87%)
4.195: X = I, R = TIPS (76%)

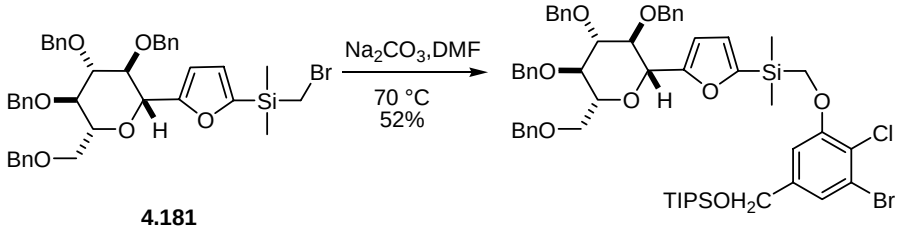
Scheme 4.63



4.186

4.196

Scheme 4.64

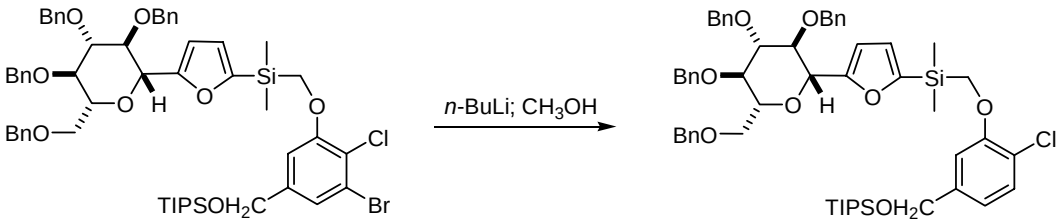


4.181

4.197

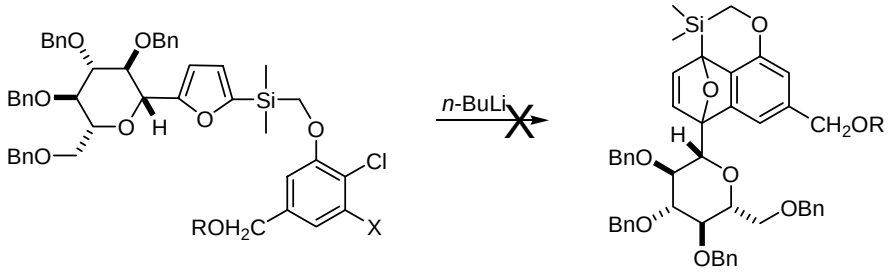
As expected, complete lithium-halide exchange took place rapidly upon treatment with *n*-BuLi at –95 ºC. Quenching the resultant organolithium with methanol at low temperature afforded the chloroanisole **4.198** (Scheme 4.65). If the aryllithium was allowed to warm up, spontaneous repulsion of the chloride should take place to deliver the requisite benzyne. However, when the THF or ether solution of **4194**, **4.195**, **4.188** or **4.196** was treated with *n*-BuLi at –78, 0°C or rt, only very small amounts of the desired cycloadducts were formed in all the cases as observed from the ^{1}H NMR of the crude material and TLC. ^{1}H NMR of the crude material also revealed that significant premature attack on the tethered silicon was taking place (Scheme 4.66 and 4.67).

Scheme 4.65



4.197 **4.198**

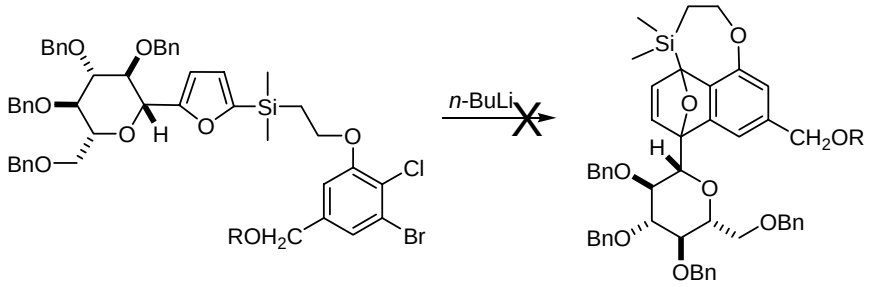
Scheme 4.66



4.194: X = Br, R = TBS
4.195: X = I, R = TIPS

4.199: R = TBS
4.200: R = TIPS

Scheme 4.67

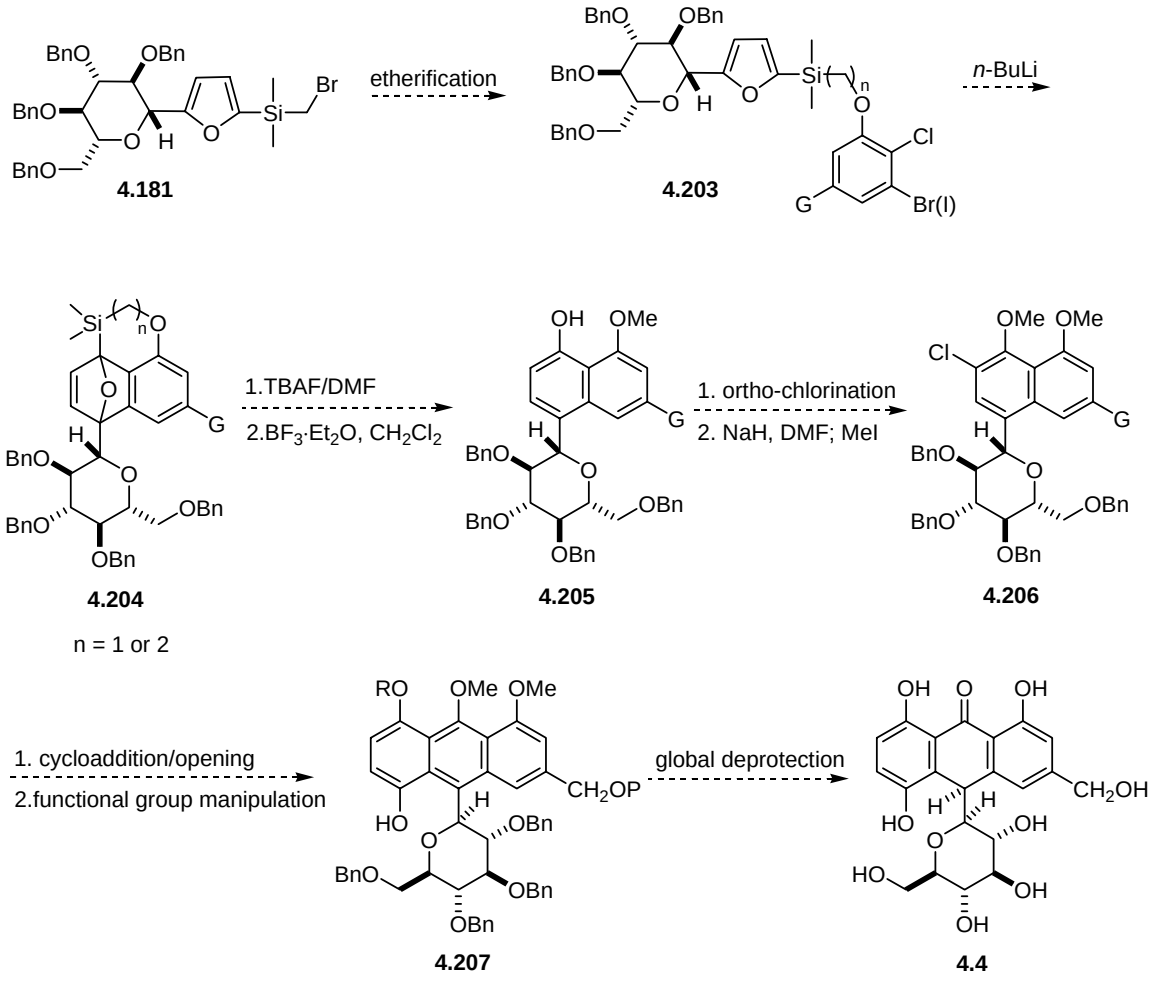


4.188: R = MOM
4.196: R = TIPS

4.201: R = MOM
4.202: R = TIPS

It became clear that our attempts to compensate the low reactivity of the benzyne **4.101** and improve the chemical yield of the cyclization by increasing the effective concentration of the glycosyl furan by ways of an intramolecular cyclization just could not deliver the desired cycloadducts in acceptable yields. Therefore, future efforts should be directed at studying the substituent effect of the benzyne in the cyclization. The success of the route shown in Scheme 4.68 will be dependent on our ability to nail down an appropriate benzyne substituent (G: a latent hydroxylmethyl group). The rest of the proposed synthesis is relatively straightforward as major transformations have been demonstrated in our current efforts.

Scheme 4.68



4.3 THE SYNTHESIS AND DERIVATIZATION OF C-ARYL GLYCAL

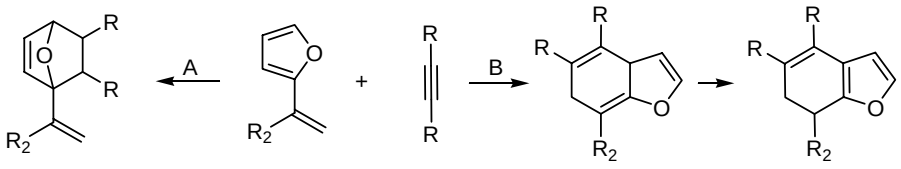
During the course of the investigation, it came to our attention that benzyne/furan cycloadducts possessing oxygenated sugars such as **4.132** were more resistant to the acid-catalyzed ring opening than those with 2-deoxy sugars such as **4.7**. Harsher conditions were typically required to cleave the oxabicyclic ring of **4.132**, and the product naphthol **4.133** underwent slow decomposition under reaction conditions. This necessitated material recycling to obtain a better yield in some cases. It was also discovered that when

a second electron-withdrawing group was present on the cycloadducts, such as **4.114**, the cycloadducts were highly resistant to acid-catalyzed ring opening and harsher condition only led to the decomposition of materials. It was thought that the acid catalyzed ring opening could be facilitated if an electron-donating functionality was present on the carbohydrate moiety, which would stabilize the developing positive charge in the transition state. Hence, the possibility of bringing in a glycal functional group was conceived, such a functional group could be readily converted in to 2-deoxy sugar and 2-oxygentaed sugars when needed.

4.3.1 Benzyne-Furan Cycloaddition of Furyl Glycal

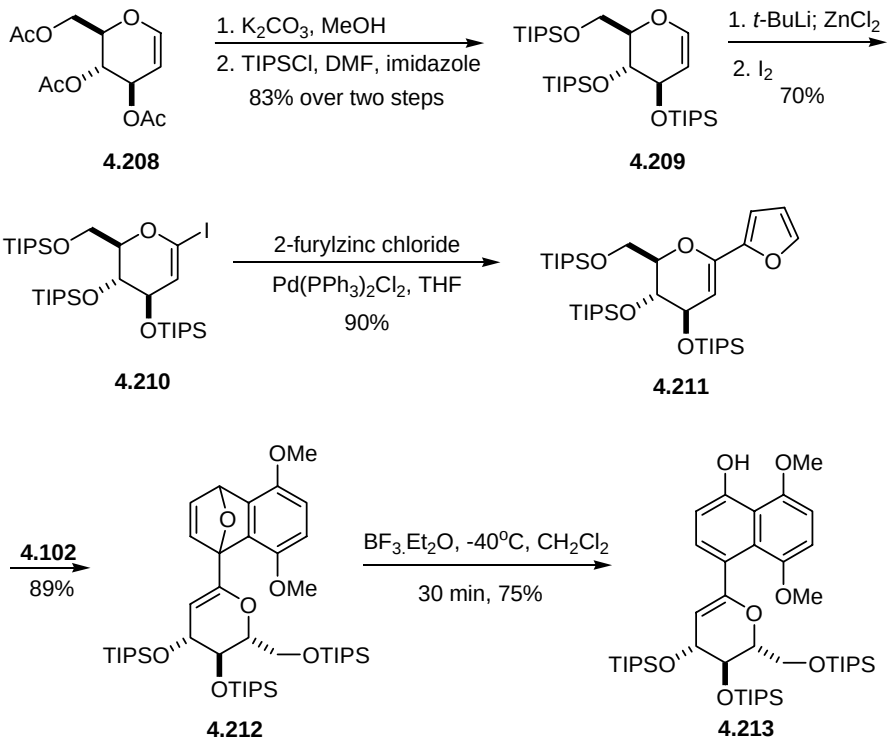
One potential problem involves the regioselectivity of bond formation in the dienic unit in the cycloaddition. Namely, two modes of cycloadditions are plausible as depicted in Scheme 4.56, and the conjugated system involving the exocyclic double bond in 2-vinyl furan was reported to be generally more reactive than the furan ring itself in the cycloadditions.[332] The reactivity pattern, however, was affected by both electronic and steric factors. For example, Kotsuki observed that a electron withdrawing acetyloxy group at R_2 decreased the reactivity of the diene system involving the exocyclic double bond and gave exclusively the product arising from path A (Scheme 4.69).[333]

Scheme 4.69



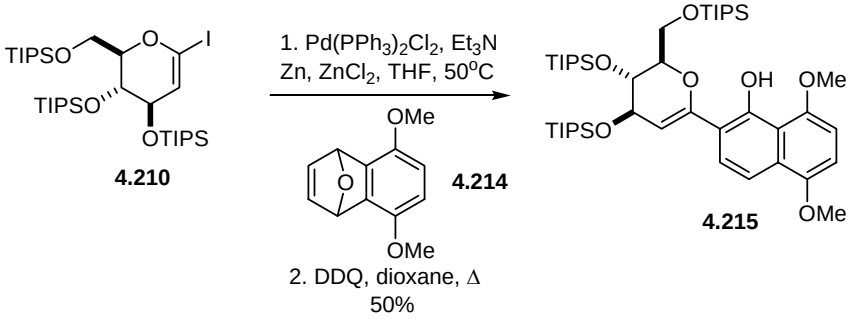
It was envisioned that by introducing steric hindrance around the *exo*-double bond in the furyl glycal, cycloaddition via path A might be favored. Thus, furyl glucal **4.211** was prepared through Negishi coupling and subjected to the cycloaddition condition.[334] We were delighted to find that the cycloaddition proceeded smoothly, and the *exo*-double bond remained intact throughout the reaction (Scheme 4.70). This is the first example of an intermolecular benzyne-furan cycloaddition in which a furyl glycal was employed as the dienic unit to form the oxabicyclic ring system. The oxa-bridge of the cycloadduct **4.212** could be opened under much milder condition than was needed for the cycloadducts possessing 2-deoxy sugars. Indeed, lower temperature and mild Lewis acidic conditions were needed to avoid significant decomposition of materials and produce a decent yield of product.

Scheme 4.70



This strategy effectively allows the access to all major classes of *C*-aryl glycosides, hence constituting a natural extension of our glycosyl furan/benzyne cycloaddition methodology. It also represented an alternative approach to glycal-substituted naphthols. Previously, Dr. Lopez has demonstrated that Group II and IV *C*-aryl glycals could be accessed through a Pd(0) catalyzed S_N2' ring opening of benzyne/furan cycloadducts (Scheme 4.71).[269] However, since the sugar substitution inevitably ends up *ortho* to the phenolic hydroxyl being generated, Group I *C*-aryl glycosides, where no sugar is ortho to phenolic hydroxyl, cannot be accessed directly through his approach.

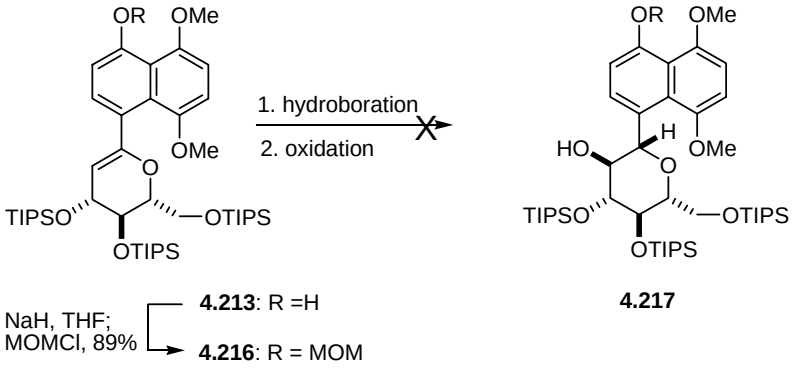
Scheme 4.71



The glycal enol ether double bond could be subsequently derivatized to afford *C*-aryl glycosides with different carbohydrate moieties. For example, hydrogenation with platinum catalyst would afford a 2-deoxysugar, while hydroboration followed by oxidation or amination would afford 2-oxygenated sugars or 2-aminosugars, respectively. Hence the free naphtholic hydroxyl was protected as its MOM ether to avoid complication in the basic peroxide oxidation step, and the **4.216** thus obtained was subjected to hydroboration. However, the hydroboration was sluggish, and large amounts

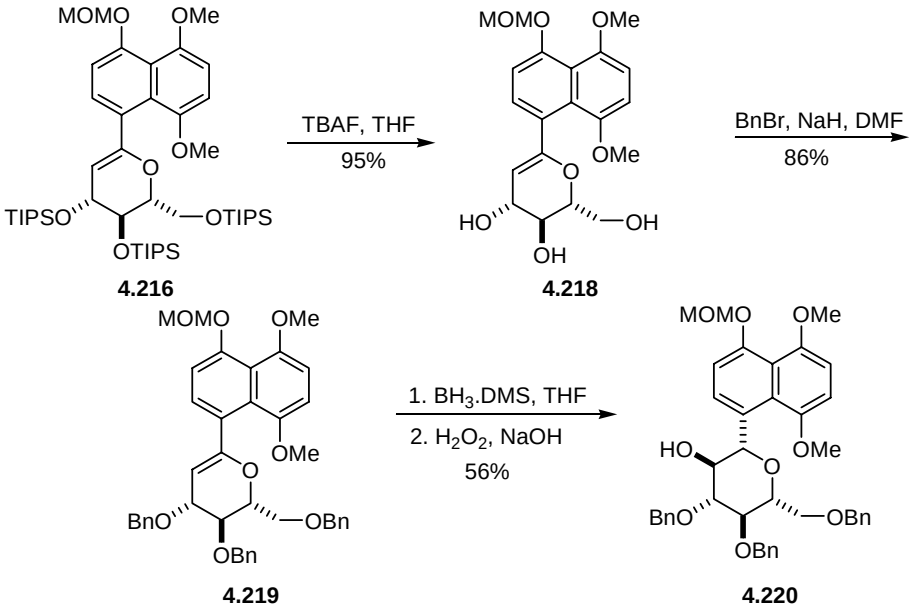
of staring material remained unreacted in the presence of large excess of BH_3·THF at elevated temperature for extended period of time (Scheme 4.72). Subsequent oxidation with basic peroxide afforded nothing identifiable in the ^1H NMR spectrum. It appeared that the very steric bulk that blessed us in the benzyne/furan cycloaddition was now working against us.

Scheme 4.72



Thus, global deprotection of the trisopropylsilyl protecting group of **4.216** with TBAF afforded **4.218**, which was then reprotected as its tribenzyl ether **4.219**. Hydroboration of **4.219** with BH_3·DMS or BH_3·THF at room temperature followed by oxidation with basic peroxide afforded the desired product **4.220** as a single diastereomer in satisfactory yield (Scheme 4.73). The β-configuration was evident by the large anomeric proton coupling constant ($J_{1,2}$ = 8.9 Hz).

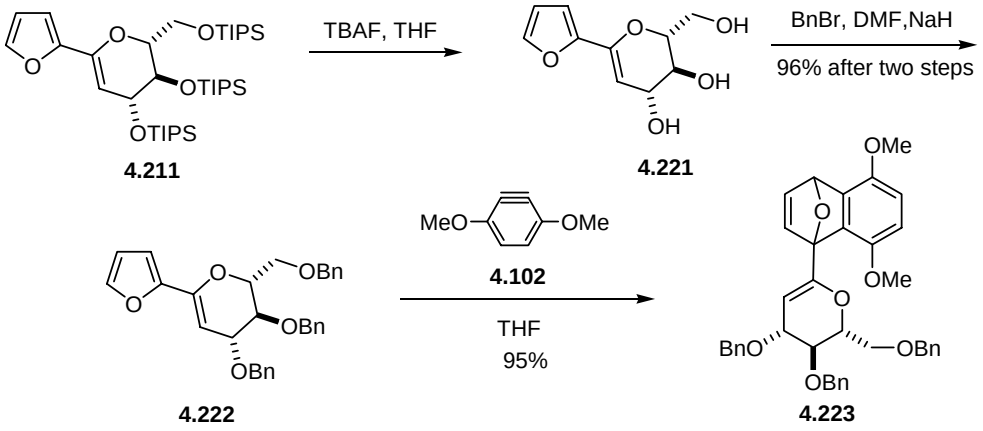
Scheme 4.73



4.216 **4.218** **4.219** **4.220**

Encouraged by this discovery, we subsequently explored the cycloaddition of the benzyne **4.102**, which was generated form 2-chloro-1,4-dimethoxybenzene (**4.103**), with the tribenzylglucal **4.222**, which was again prepared by deprotection and reprotection of its TIPS counterpart **4.211**. To our pleasure, the cycloaddition proceeded smoothly to afford the desired cycloadduct **4.223** as a mixture (1:1) of stereoisomers in excellent yield (Scheme 4.74).

Scheme 4.74



Thus far, we had cleared two major obstacles in our route to *C*-naphthyl glycosides. We have demonstrated that the cycloaddition of the selected benzyne **4.102** with benzyl protected furyl glycal can occur in a regioselective manner to afford the expected cycloadducts. The subsequent hydroboration/oxidation proceeded in regio- and stereo-specific manner to provide the desired product. At this stage, the two remaining problems to a successful method were to streamline the synthesis of the staring dienic partner **4.222** and to open the oxabicycle **4.223** in a regiocontrolled fashion.

4.3.2 Synthesis of Furyl Glycals

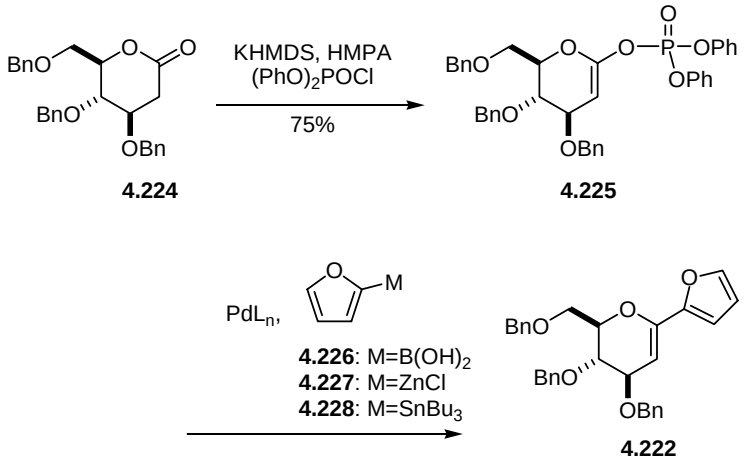
The tribenzylglucal **4.222** was previously prepared from the TIPS counterpart **4.211** after protecting group swap. Consequently, its synthesis was rather clumsy even though it had provided an easy access to the requisite material at that time. However to establish the value of the method, an overhaul of the synthesis was mandated. It was however not possible to apply the synthetic strategy used to prepare **4.211** shown in Scheme 4.70, which was prepared from the corresponding iodoglucal **4.210**. The preparation of iodoglucal required large excess of *t*-BuLi to generate the vinyl

glycosyllithium intermediate,[230, 335] and the benzylic protons will be competitively lithiated in the process. Attempted direct lithiation of benzyl-protected glycals has been reported to be unsuccessful in earlier work.[336-338]

Researchers have prepared functionalized glycals to perform cross couplings with aryl organometallic species to access *C*-aryl glycols by a number of methods. For instance, Nicolaou prepared cyclic ketene acetal phosphates from the corresponding sugar lactones and successfully used them in Stille couplings with vinylstannanes.[339, 340] Sasaki reported a Suzuki cross-coupling between an eight-membered ketene acetal phosphate and a seven-membered alkylborane in his synthesis of HIJK ring model of ciguatoxin.[341] Coudert reported the Suzuki cross-coupling of aryl boronic acids with vinyl phosphates derived from lactams.[342]

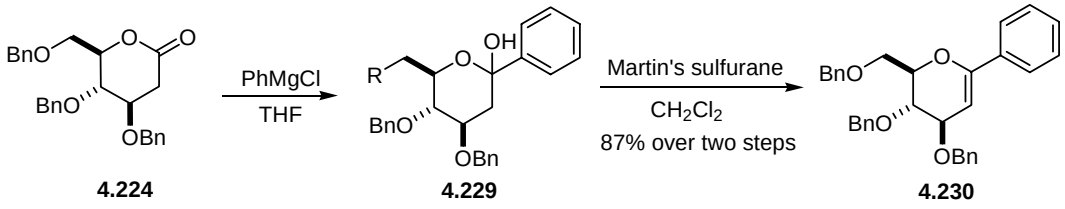
Gabrielle Kolakowski, a graduate student in the Group, examined the reaction between ketene acetal enol phosphate **4.225**[339] and metallofurans under a number of coupling reaction conditions (Scheme 4.75). Unfortunately, attempted Suzuki, Negishi and Stille couplings of phosphate **4.225** with metallofurans produced the desired *C*-aryl glucal **4.222** in only low yields (<33%).[343]

Scheme 4.75



Aryl glycals have also been prepared from its corresponding lactones on several occasions. Sulikowsk[182] reported earlier that hemiketal **4.229** could undergo dehydration with Martin's sulfurane to produce phenylglucal **4.230** in good yield (Scheme 4.76). McDonald demonstrated that $POCl_3$ could effect the dehydration of the hemiacetal generated from addition of 2-(trimethylsily1)ethynylmagnesium bromide to a *tert*-butyldiphenylsilyl protected 2-deoxy-D-gluconolactone, albeit in low yield.[265] Because Sulikowsk's optimized conditions required five equivalents of the expensive and unstable Martin's sulfurane, this approach was hardly practical. The dehydrations were performed at room temperature in a separate step, so the hemiketal intermediate **4.229** must stay in closed form throughout the dehydration to assure reasonable yields. However, the hemiketal **4.232**, obtained from addition of 2-lithiofuran to 2-deoxygluconolactone **4.224**, existed predominantly as the open form **4.231a** at room temperature in $CDCl_3$, and a number of other hemiacetals prepared in the group in the past existed as mixtures of open and closed forms. Understandably, attempts to obtain the 2-furylglucal **4.222** from lactone **4.224** using Sulikowsk's procedure only gave poor yield (32%).

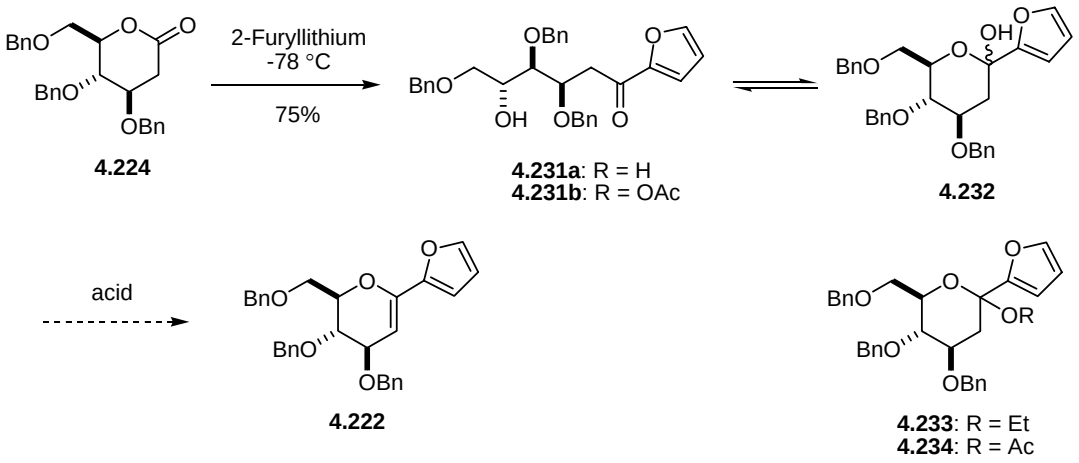
Scheme 4.76



It was envisioned that the acyclic form of the lactol **4.231a** might cyclize and undergo dehydration under acidic conditions to afford glucal **4.222** (Scheme 4.77). However, when Gabrielle Kolakowski subjected the 5-hydroxy ketone **4.231a** to a variety

of Brønsted and Lewis acids, only complex product mixtures were obtained. Hence, attempts were made to effect the dehydration of **4.231a** in a stepwise manner incorporating a separate cyclization step to form the cyclic acetal **4.233**. Exposure of ethyl cyclic acetal **4.233** to Brønsted and Lewis acid in the presence of a non-nucleophilic base did not provide Kolakowski any of the desired **4.222**.[343] In the case of cyclic ethyl ketal **4.233**, the generation of the oxocarbenium may require a stronger acid than what could be tolerated by the product **4.222**. Therefore a leaving group better than alkoxy group would be desirable.

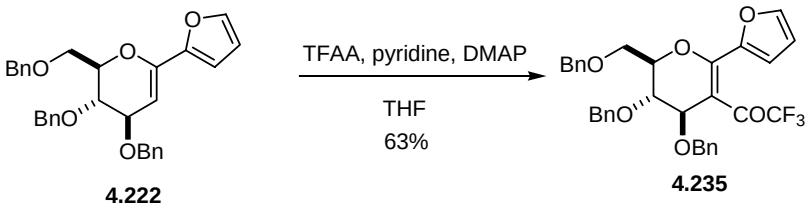
Scheme 4.77



Seeing that attempt to generate the glucal **4.222** from **4.231a** was unsuccessful, we decided to trap the lithium alkoxide of the lactol before it opened. Thus, treatment of the alkoxide, which was generated by the action of 2-lithiofuran on the lactone **4.224,** with acetic anhydride under Rychnovsky's conditions (DMAP/pyridine/Ac_2O)[344] did not yield the acetylated lactol **4.234**. Rather the glucal **4.222** was obtained in 19% yield along with large amount of acetylated 5-hydroxylketone **4.231b**.[344, 345] It was reasonable to assume that the glucal was formed from the acylated lactol **4.234**, and so the collapse of

lithium alkoxide of the lactol was competing with the acetylation of the tertiary alcohol under the reaction conditions. The occurrence of the unfavorable competition was not unexpected since we were attempting to trap a hindered tertiary alkoxide. Use of an electrophile better than acetic anhydride would be expected to accelerate the rate of the acylation so that it could be faster than the rate of the ring-opening of the lithium alkoxide of the lactol. Hence trifluoroacetic anhydride (TFAA) was employed as the trapping reagent. Switching to TFAA (4 equivalent) nearly doubled the yield of the desired arylglucal **4.222**; however, a major impurity, which was identified as the acylated glucal **4.235**, was also formed. The acylated glucal **4.235** was formed through electrophilic acylation of the enol ether (Scheme 4.78). Similar transformations have been reported by Hojo in the preparation of β-alkoxy-α,β-unsaturated acids from enol ethers.[346]

Scheme 4.78



4.222 **4.235**

To avoid the problem of over-acylation, the stoichiometry of TFAA was reduced to 2.5 equivalents, and the furylglucal **4.222** was obtained in an improved yield of 75%. Methanesulfonyl chloride and triflic anhydride were also used as electrophiles to trap the lithium alkoxide of the lactol **4.232**, but they were found not to be as effective as TFAA.

Hence, we have discovered that lithium alkoxide **4.237** that was generated by addition of 2-lithiofuran to the 2-deoxygluconolactone **4.224** could be trapped *in situ* with trifluoroacetic acid anhydride (TFAA) at low temperature before it opened to form 5-

hydroxyketone **4.231a** (Scheme 4.79). Elimination of the trifluoroacetate of **4.238** afforded the furylglucal **4.222** in good yield. In the presence of large excess TFAA, the glucal **4.222** could be further acylated at 2-position leading to 1-furyl-2-trifluoroacetoxyglucal **4.235** as a common byproduct. Should **4.235** be the desired product, a one-pot transformation from the sugar lactone **4.224** could be developed.

Scheme 4.79

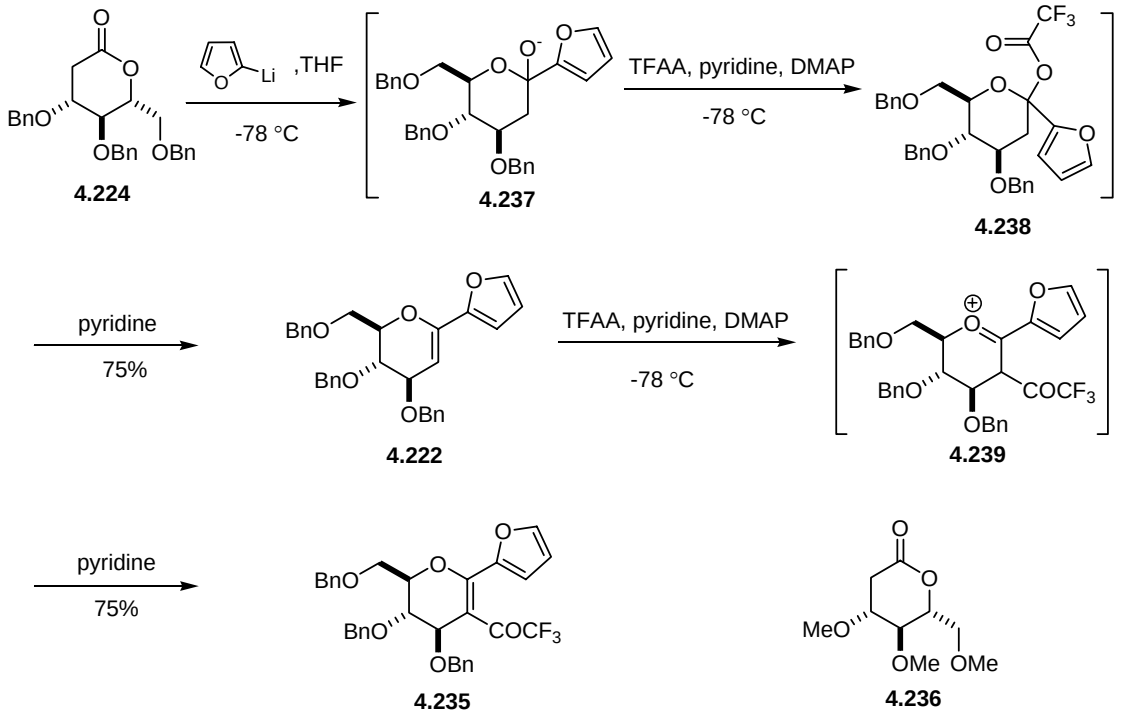


Table 4.3: Partial Optimization in Converting Sugar Lactone to Furylglucal

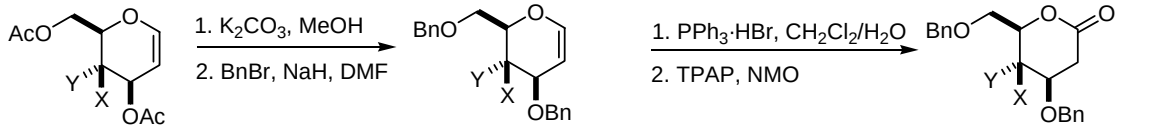
	Acylation/Sulfonation Reagent	Equivalent	Observation and yield
1	**4.224**, AcCl	4	19% of **4.222** and acylated ring-opened product
2	**4.224,** TFAA, $ZnCl_2$ (1.2 mol)	4	12% of **4.222** and 25% of **4.235**
3	**4.224,** TFAA	4	35% of **4.222** and 22% of **4.235**
4	**4.224,** MsCl	4	32% of **4.222**
5	**4.224,** TFAA	1.5	57% of **4.222**
6	**4.224,** TFAA	2.5	75% of **4.222**
7	**4.224,** TFAA (reverse addition)	2.5	49% of **4.222**

8	**4.224,** TFAA	3.0	66% of **4.222** and trace of **4.235**
9	**4.236,** Tf_2O	2.5	13% of **4.252**
10	**4.236,** TFAA	2.5	64% of **4.252**

* All reactions were run in THF at -78°C, pyridine (3 mol), DMAP (1 mol), and a solution of furyllithium was added to a solution of lactone at –78 °C.

Further exploration revealed that a variety of aryllithium reagents could be added to the known sugar lactones **4.224**, **4.198**, **4.243** and **4.246** to obtain generally good to excellent yields of the corresponding aryl glycals (Table 4.4). The lactones were prepared from the corresponding commercially available peracetoxy glycals according to established procedures,[272, 347] involving saponification of the acetoxy groups and reprotection as the benzyl ethers followed by hydration of the glycal with PPh_3·HBr catalyst and subsequent oxidation of the lactol with TPAP (Scheme 4.80 and 4.81).

Scheme 4.80

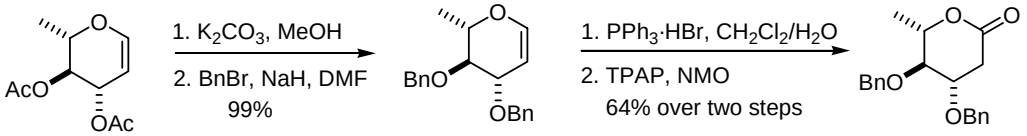


4.208: X = H, Y = OAc
4.240: X = OAc, Y = H

4.241: X = H, Y = OBn (85%)
4.242: X = OBn, Y = H (55%)

4.224: X = H, Y = OBn (72%)
4.243: X = OBn, Y = H (96%)

Scheme 4.81



4.244 **4.245** **4.246**

As shown in Table 4.4, the general trend in this transformation was that rhamnals were formed in the best yields in all the case. The formation of galactals proved to be problematic, and significant quantities of unidentified impurities were always isolated

(Entry 7). The acetylation of the alkoxide of the lactol derived from galactal might have been slow. The galactal derivatives also appeared to be less stable than other aryl glycals, which might be a contributing factor in the lower yield. When alkyllithium and alkylmagnesium reagents were used (Entry 9 and entry 10), only the *endo*-glycal was obtained; no *E-exo* or *Z-exo* glycals were detected.[348, 349] However, the chemical yields in these cases are too low for this method to be synthetically useful.

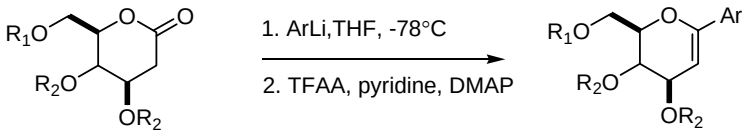
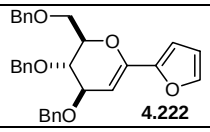
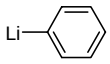
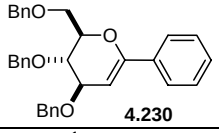
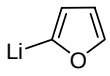
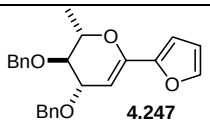
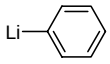
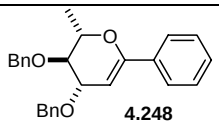
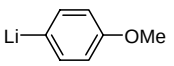
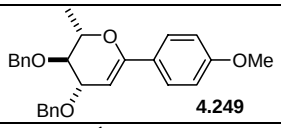
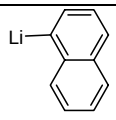
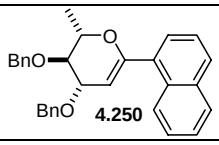
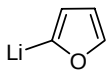
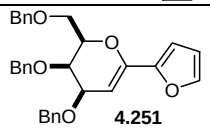
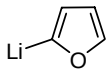
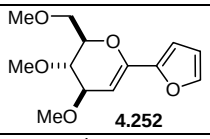
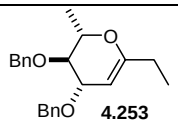
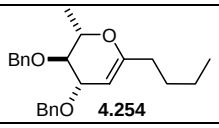


Table 4.4: Partial Optimization of Converting Sugar Lactone to Furylglycal

Entry	Substrate	organometal	Product	Yield
1	**4.224**		 **4.222**	75%
2	**4.224**		 **4.230**	60%
3	**4.246**		 **4.247**	93%
4	**4.246**		 **4.248**	91%
5	**4.246**		 **4.249**	80%
6	**4.246**		 **4.250**	95%
7	**4.243**		 **4.251**	31%

8	**4.236**		 **4.252**	64%
9	**4.246**	EtMgCl	 **4.253**	19%
10	**4.246**	BuLi	 **4.254**	36%

The transformation above represents an alternate approach to prepare the *C*-aryl glycals starting with the corresponding 2-deoxysugar lactones. Furyl glycals prepared thus way can be directly used in our glycosylfuran/benzyne cycloaddition methodology.

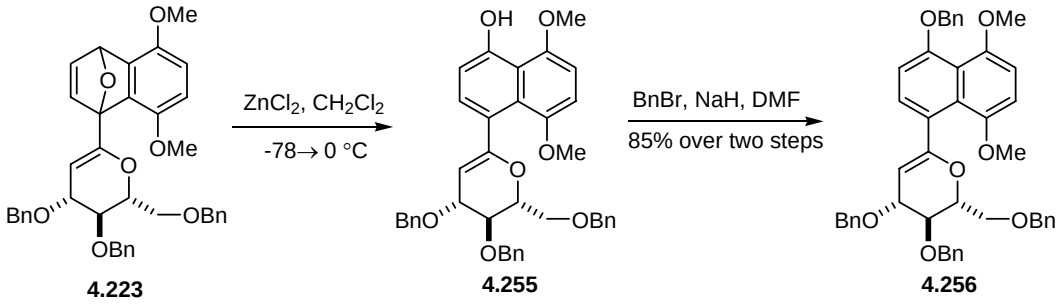
4.3.3 The Acid Catalyzed Rearrangement of Oxabicycles With Glycal Substituent

The cycloadducts possessing glycal substituents have shown to undergo facile ring opening and simultaneous rapid decomposition when exposed to Brønsted and Lewis acids. The cycloadduct **4.223** and the corresponding naphthol **4.255** are significantly more sensitive towards acids than their triisopropylsilyl protected counterpart **4.212** and **4.213**, probably due to more exposed enol ether double bond. Condition previously used to open cycloadduct **4.212** ($BE_3{\cdot}Et_2O$, 2,6-lutidine, -5 °C) only led to a mixture of multiple phenols.

Kristen Procko, another graduate student in the group, examined the use of a number of Lewis acids on the opening of oxabicycle **4.223** and she discovered that although zinc chloride did not effect ring opening at –78 ºC, warming up the mixture to 0 ºC led cleanly to the desired naphthol **4.255** (Scheme 4.82). If the reaction was conducted

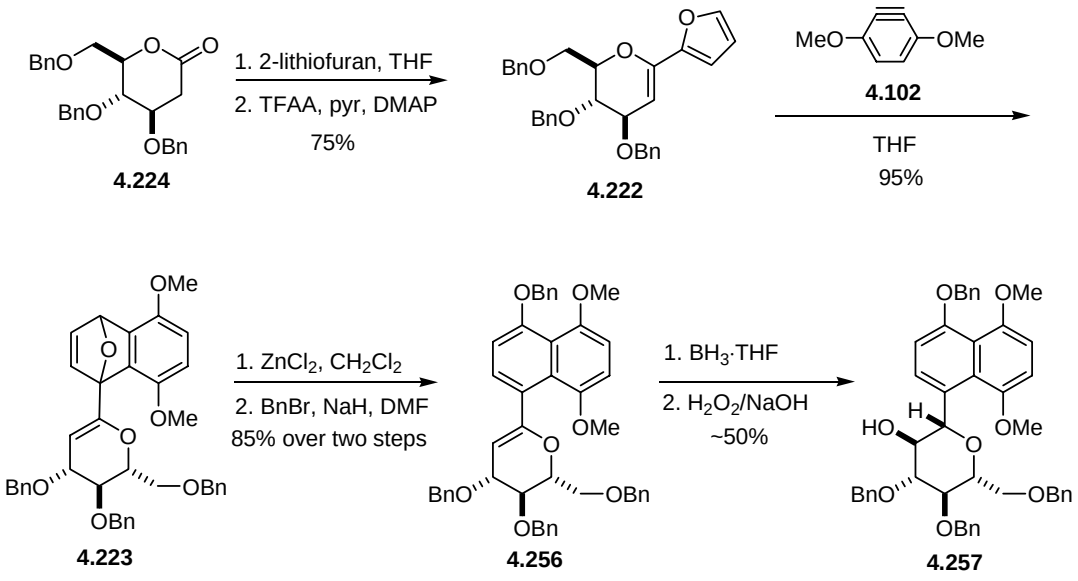
at 0 ºC, multiple compounds were formed. It was thus necessary that all the components were mixed at a lower temperature.[350]

Scheme 4.82



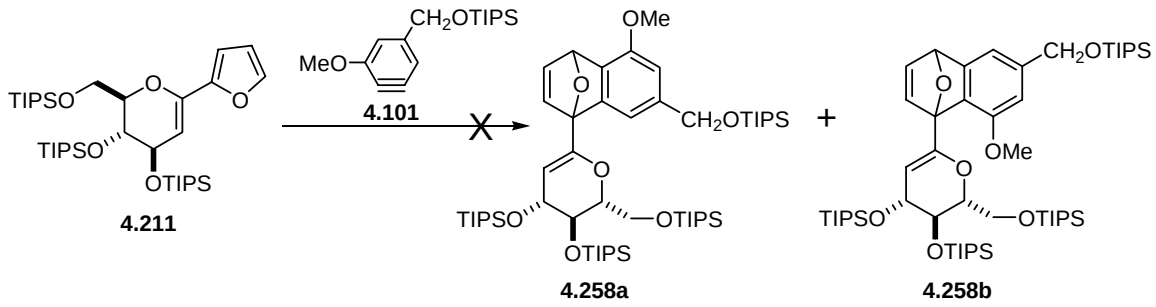
The success in the opening of the oxabicycle **4.223** hence cleared the final hurdle in the sequence, establishing the feasibility of our approach to *C*-naphthyl glycosides containing 2-deoxy and 2-oxy carbohydrate moieties (Scheme 4.83)

Scheme 4.83



Though the above discussed method could be applied in the synthesis of 5-hydroxyaloin A, at this moment, clear advantage of bringing in the glucal as a latent oxygenated carbohydrate is lacking. The furyl glucal **4.211** has behaved quite similarly to glycosyl-substituted furans, and cycloaddition with benzyne **4.101** failed to deliver the desired cycloadducts and the furyl glucal **4.211** was recovered (Scheme 4.84). Bringing in an extra enol ether function functionality into the synthesis of 5-hydroxyaloin A therefore appeared to only add extra steps to the sequence.

Scheme 4.84



4.4 CONCLUSION

In the application of our glycosyl furan/benzyne cycloaddition methodology towards natural product synthesis, a two-stage benzyne/furan cycloaddition strategy was used to assemble the anthrone core of Group I *C*-aryl glycoside 5-hydroxyaloin A. Proof of concept was established in the generation of benzyne from a chloronaphthol precursor **4.142** and subsequent cycloaddition with furan afford the cycloadduct **4.143** (Scheme 4.53). However, the cycloadducts of **4.142** and alkoxy/silyloxy furans were unstable, and attempts to convert them into 5-hydroxyaloin A were unsuccessful. During the course of the investigation, the scope and limitation of the benzyne/furan cycloaddition was examined, and it was found that the second methoxy group on the benzyne had a

tremendous effect on its reactivity as the dienophile; Lack of this methoxy resulted in the low reactivity of benzyne **4.101** in the cycloaddition. Unfortunately, attempts to optimize the cyclization by increasing the effective concentration of glycosyl furan by ways of an intramolecular cyclization failed to deliver the cycloadducts in acceptable yields.

During the course of the investigation, an approach to the *C*-naphthyl glycosides featuring a benzyne/furan cycloadditiont using a glycal-substituted furan was developed. The derivatization of the glycal double bond allows us to access *C*-aryl glycoside containing 2-deoxy and 2-oxygenated sugars from a common intermediate, including members from Group I *C*-aryl glycoside, A novel approach to *C*-aryl glycals was established during the course. This transformation allows us to access *C*-aryl glycals from the corresponding deoxy sugar lactones after a single step.

Chapter 5: Experimental Procedures

5.1 MATERIALS

The phospholipid substrate 1,2-dihexanoyl-sn-glycero-3-phosphocholine (C6PC) was purchased from Avanti Polar Lipids (Alabaster, AL). Alkaline phosphatase (P-5521), 3,3-dimethylglutaric acid (DMG), bovine serum albumin (A-3059), and PLCBc (P-7147) were purchased from Sigma (St. Louis, MO). Ammonium molybdate tetrahydrate, L-ascorbic acid, and sodium metaarsenite were obtained from Aldrich Chemical Co. (Milwaukee, WI). Trichloroacetic acid and sodium citrate were obtained from EM Science (Gibbstown, NJ). The kinetic assays were performed in 96-well plates obtained from Fisher Scientific. The microplate reader used was a SpectraMax 340 from Molecular Devices.

5.2 METHODS FOR INORGANIC PHOSPHATE QUANTITATION ASSAY

The specificity constants K_{cat}/K_M of PLC_{Bc} were determined using a sensitive, enzyme-coupled assay that is based on the quantitation of Pi.[284] In short, the cholinephosphate produced by the PLC-catalyzed hydrolysis of C6PC was treated with alkaline phosphatase to liberate Pi, which formed a complex with ammonium molybdate. The complex was then reduced to a blue molybdenum state with ascorbic acid to yield a blue solution with a maximum absorbance at 700 nm. The initial velocity versus substrate concentration data were obtained in at least duplicate in the presence and absence of inhibitor at substrate concentrations well below their CMCs in the assay buffer. The specificity constants, K_{cat}/K_M, were obtained from the slopes of the initial velocity

versus substrate concentration curves at low substrate concentrations, and the K_i for each inhibitor was determined from these data using equation 3.14

5.3 EXPERIMENTAL

5.3.1 General

Unless otherwise noted, solvents and regents were used without purification. Methylene chloride (CH_2Cl_2) was distilled from calcium hydride prior to use. Tetrahydrofuran (THF) was dried by passage through two column of activated neutral alumina. *N, N*-dimethylformamide (DMF) was dried by passage through two columns of activated molecular sieves. Acetonitrile (MeCN), diethyl ether (Et_2O) and methanol (MeOH) were passed over molecular sieves prior to use. All solvents were deemed to contain less than 50 ppm H_2O by Karl Fischer coulomeric moisture analysis. Reactions involving air or moisture sensitive reagents or intermediates were performed under an inert atmosphere of argon in glassware that had been oven or flame dried. Reagents were purchased from Aldrich and used without furthur purification unless indicated otherwise. Chemical abbreviations used are as follows: borane-tetrahydrofuran complex (BH_3·THF), Boron trifluoride etherate (BF_3·Et_2O), *tert*-butyldimethylsilyl chloride (TBDMSCl), chloromethyl methyl ether (MOMCl), 2,6-dichloro-3,5-dicyanoquinone (DDQ), diisopropylamine (iPr_2NH), dicyclohexylcarbamide (DCC), ethyl acetate (EtOAc), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), *N, N*-dimethylaminopyridine (DMAP), hexachloroethane (C_2Cl_6), tetrabutylammonium fluoride (TBAF), trifluroacetic anhydride (TFAA), trifluoroacetic acid (TFA).

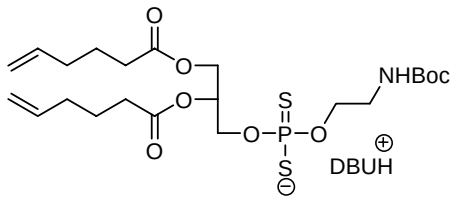
Thin-layer chromatography (TLC) was performed on EM 250 micro silica gel plates. The plates were visualized by staining with AMCAN (ammonium

molybdate/cerium ammonium nitrate), or potassium permagnate. Flash chromatography was performed with ICN Silica gel 60 according to established protocol.[351]

The ^{1}H, ^{13}C NMR's were obtained on a Varian 500 series, Varian MERCURY 400, or a Bruker AM-250 spectrometer, operating at 500 (400, 250) and 125 (100, 62) MHz respectively. Unless indicated otherwise, all spectra were run as solution in $CDCl_3$. For ^{1}H NMR, chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane (TMS) and are, in all cases, referenced to the residual protio-solvent present: δ 7.24 for $CHCl_3$, δ 7.15 for benzene-d_6, δ 3.30 for the methyl peak of CD_3OD, 2.04 for acetone-d_6. In ^{13}C NMR, chemical shifts were reported in ppm relative to the center line of the multiplet for deuterium solvent peaks: δ 77.0 (t) for $CDCl_3$, δ 128.0 (t) for benzene-d_6, δ 49.0 (septet) for the methyl peak of CD_3OD, δ 29.8 (septet) for acetone-d_6. ^{13}C spectra were routinely run with broadband ^{1}H decoupling. Coupling constants for all spectra are reported in Hertz (Hz).

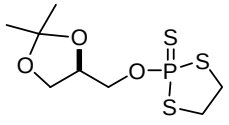
Infrared spectra were measured on a Perkin Elmer 1600 FTIR spectrometer either neat or as solutions in the indicated solvent on sodium chloride plates and are recorded in units of cm-1. Low-resolution chemical ionization (CI) mass spectra were performed on Finnigan MAT TSQ-70 instrument; HRMS were made with a VG analytical ZAB2-E instrument.

5.3.2 Compounds



3.13a

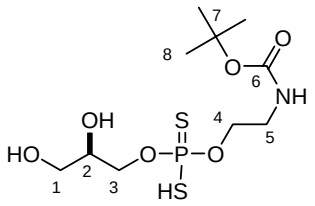
***N*-*tert*-Butylcarbonyl-1,2-di-*n*-(5-hexenoyl)-*sn*-glyceryl-3-phosphodithio-ethanolamine (3.13a)**. To a solution of **3.11a** (170 mg, 0.39 mmol) and *N*-Boc-ethanolamine (60 μL, 0.39 mmol) in dry CH_3CN (5 mL), was added DBU (58 μL, 0.39 mmol) dropwise at rt. The solution was stirred at rt overnight. The solution was filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by chromatography eluting with acetone/$CHCl_3$/H_2O (67:32:1) to yield 220 mg (82%) of **3.13a** as a yellow liquid. (Due to the presence of DBU, this compound was not characterized**)**



3.14

1,2-Di-*O*-isopropylidene-*sn*-3-glyceryl-2-thio-1,3,2-dithiophospholane (3.14). To a solution of **1.1** (820 mg, 6.2 mmol) and diisopropylethylamine (880 mg, 6.8 mmol) in CH_3CN (52 mL) at –38 °C (dry ice/1,2-dichloroethane) was added 2-chloro-1,3,2-dithiophospholane dropwise (980 mg, 6.2 mmol) . The solution was stirred at –38 °C for 2 h. The cold bath was removed and the mixture was stirred for another 60 min. A solution of sulfur (620 mg, 19.4 mmol) in CS_2 (16 mL) was added in one portion at rt. The solution was stirred vigorously for 7 h. The mixture was then concentrated under reduced pressure and the residue was dissolved in acetone. The insoluble solid was removed by filtration and washed with acetone. The combined filtrate and wash were concentrated under reduced pressure. The residue was purified by chromatography eluting with $CHCl_3$ to yield 1.534 g (86%) of **3.14** as a pale yellow oil: ^{1}H NMR (250 MHz) δ 4.29 (p, *J* = 5.6 Hz, 1 H), 4.07-3.99 (comp, 3 H), 3.77 (dd, *J* = 8.6, 5.5 Hz, 1 H),

3.67-3.54 (comp, 4 H), 1.36 (s, 3 H), 1.29 (s, 3 H); ^{13}C NMR (62 MHz) δ 109.8, 73.7 (d, J = 9.9 Hz), 67.6 (d, J = 9.3 Hz), 66.08, 41.4 (d, J = 5.3 Hz), 26.6, 25.2; ^{31}P NMR (121 MHz) δ 123.7.

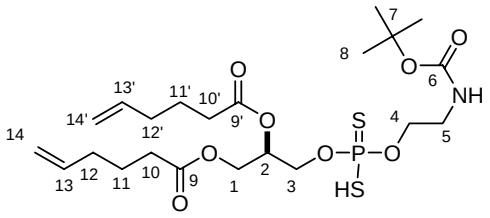


3.16

***N-tert*-Butoxylcarbonyl-*sn*-glyceryl-3-phosphodithioethanolamine (3.16).** To a solution of **3.14** (260 mg, 0.90 mmol) and *N*-Boc-ethanolamine (165 mg, 1.02 mmol) in dry CH_3CN (10 mL), was added DBU (155 μL, 1.02 mmol) dropwise at rt. The solution was stirred for 40 min and the solvent removed under reduced pressure. The residue was purified by column chromatography over silica gel, eluting with acetone/$CHCl_3$ (2:1) to yield 487 mg (99%) of **3.15** as yellow oil.

The **3.15** (114 mg, 0.21 mmol) thus obtained was dissolved in t-BuOH/AcOH (2 mL, 1:3), and the solution was stirred overnight at rt. The solvent was removed under reduced pressure, and the residue was purified by flash chromatography eluting with acetone/$CHCl_3$ (2:1) to yield 45 mg (61%) of **3.16** as a glass: ^{1}H NMR (300 MHz, acetone-d_6) δ 6.18 (s, 1 H), 4.72-4.50 (br, s, 1 H), 4.50-4.15 (br, s, 1 H), 4.10-3.87 (comp, 5 H), 3.70-3.61 (comp, 2 H), 3.31(dd, J = 9.0, 5.4 Hz, 2 H), 1.39 (s, 9 H); ^{13}C NMR (75 MHz, acetone-d_6) δ 156.6, 78.5, 71.1 (d, J = 7.1 Hz), 66.4 (d, J = 7.1 Hz), 64.6 (d, J = 6.0 Hz), 63.0, 41.2 (d, J = 7.1 Hz), 28.1; ^{31}P NMR (121 MHz, acetone-d_6) δ 118.2; IR ($CHCl_3$) 3562, 3308, 2973, 1693, 1646, 1520, 1453 cm^{-1}; mass spectrum (CI) *m/z* 348.0703 [$C_{10}H_{23}NO_6PS_2$ (M+1) requires 348.0704], 282, 258, 199, 185 (base).

NMR assignments. ^{1}H NMR (300 MHz, acetone-d_6) δ 6.18 (s, 1 H, S-H), 4.72-4.50 (br, s, 1 H, C2-OH), 4.50-4.15 (br, s, 1 H, C1-OH), 4.10-3.87 (comp, 5 H, C1-H & C2-H & C3-H), 3.70-3.61 (comp, 2 H, C4-H), 3.31 (dd, J = 9.0, 5.4 Hz, 2 H, C5-H), 1.39 (s, 9 H, C8-H); ^{13}C NMR (75 MHz, acetone-d_6) δ 156.6 (C6), 78.5 (C1), 71.1 (d, J = 7.1 Hz, C2), 66.4 (d, J = 7.1 Hz, C3), 64.6 (d, J = 6.0 Hz, C4), 63.0 (C7), 41.2 (d, J = 7.1 Hz, C5), 28.1 (C8);

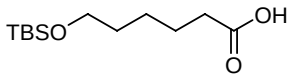


3.17

***N*-*tert*-Butoxylcarbonyl-1,2-di-*n*-(5-hexenoyl)-*sn*-glyceryl-3-phosphodithio-ethanolamine (3.17)**. To a solution of diol **3.16** (17 mg, 0.049 mmol) in dry pyridine (0.3 mL), was added 5-hexenoyl chloride (13 mg, 0.098 mmol) dropwise at 0 °C. The mixture was stirred at rt for 2 h. The solution was diluted with CH_2Cl_2 (5 mL) and washed with saturated $NaHCO_3$ (5 mL), 1 N HCl (5 mL), H_2O (5 mL), dried (Na_2SO_4), and filtered. The solvent was removed under reduced pressure, and the residue was purified by chromatography eluting with acetone/$CHCl_3$ (2:1) to yield 22 mg (82%) of **3.17** as a pale yellow liquid: ^{1}H NMR (300 MHz) δ 5.86-5.70 (m, 2 H), 5.42-5.20 (comp, 2 H), 5.08-4.96 (comp, 4 H), 4.41 (dd, J = 12.0, 3 Hz, 1 H), 4.24 (dd, J = 12.0, 6.6 Hz, 1 H), 4.16-3.98 (comp, 4 H), 3.50-3.00 (comp, 3 H), 2.37 (q, J = 7.7 Hz, 4 H), 2.12-2.02 (comp, 4 H), 1.80-1.68 (comp, 4 H), 1.45 (s, 9 H); ^{13}C NMR (75 MHz) δ 173.5, 156.9, 137.0, 115.1, 115.0, 79.7, 70.2 (d, J = 8.5 Hz), 65.8, 63.5, 62.3, 40.5, 33.1 (d, J = 15.2 Hz), 32.5

(d, J = 2.1 Hz), 28.0, 23.5, 23.4; ^{31}P NMR (121 MHz) δ 115.8; mass spectrum (CI) *m/z* 540.1825 [$C_{22}H_{39}NO_8PS_2$ (M+1) requires 540.1854], 496, 422, 267 (base), 171.

NMR assignments. ^{1}H NMR (300 MHz) δ 5.86-5.70 (m, 2 H, C13-H & C13'-H), 5.42-5.20 (comp, 2 H, C2-H & N-H), 5.08-4.96 (comp, 4 H, C14-H & C14'-H), 4.41 (dd, J = 12.0, 3 Hz, 1 H, C1-H), 4.24 (dd, J = 12.0, 6.6 Hz, 1 H, C1-H), 4.16-3.98 (comp, 4 H, C4-H & C3-H), 3.50-3.00 (comp, 3 H, C5-H & S-H), 2.37 (q, J = 7.7 Hz, 4 H, C12-H & C12'-H), 2.12-2.02 (comp, 4 H, C10-H & C10'-H), 1.80-1.68 (comp, 4 H, C11-H & C11'-H), 1.45 (s, 9 H, C8-H); ^{13}C NMR (75 MHz) δ 173.5 (C9 & C9'), 156.9 (C6), 137.0 (C13 & C'13), 115.1 (C14), 115.0 (C'14), 79.7 (C2), 70.2 (d, J = 8.5 Hz, C1), 65.8 (C3), 63.5 (C4), 62.3 (C7), 40.5 (C5), 33.2 (C12), 33.0 (C12'), 32.5(C10), 32.4 (C10'), 28.0 (C8), 23.5 (C11), 23.4 (C11').

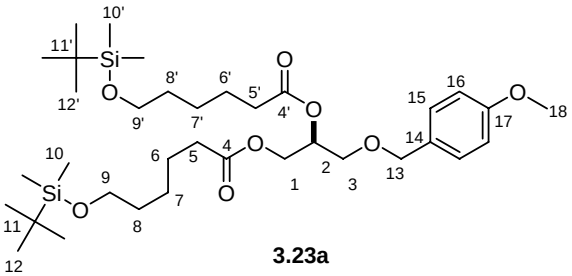


3.21a

6-*tert*-Butyldimethylsilyloxyhexanoic acid (3.21a). A solution of ε-caprolactone (4.0 g, 35 mmol) and conc. HCl (7 drops) in MeOH (100 mL) was heated at reflux for 12 h. The solvent was removed under reduced pressure. The residue was partitioned between EtOAc (60 mL) and $NaHCO_3$ (50 mL), and the organic phase was washed with brine (50 mL), dried (Na_2SO_4), and filtered. The solvent was removed under reduced pressure, and the residue was purified by distillation (77~81°C/0.06 mmHg) to yield 4.56 g (89%) of **3.19a** as clear liquid: ^{1}H NMR (250 MHz), δ 3.54 (s, 3 H), 3.50 (t, J = 6.5 Hz, 2 H), 2.42 (s, 1 H), 2.20 (t, J = 7.4 Hz, 2 H), 1.59-1.40 (comp, 4 H), 1.33-1.20 (comp, 2 H); ^{13}C NMR (75 MHz) δ 174.1, 62.2, 51.3, 33.8, 32.0, 25.1, 24.4.

To a solution of TBDMSCl (2.69 g, 17.5 mmol) in CH_2Cl_2 (34 mL) at 0 °C, was added DBU (2.88 g, 18.9 mmol) and then **3.19a** (2.34 mg, 15.8 mmol). The mixture was stirred at rt for 2 h. The solution was washed with H_2O (20 mL), 0.1 N HCl (20 mL), $NaHCO_3$ (20 mL), dried (Na_2SO_4), and filtered. The solvent was removed under reduced pressure, and the residue was purified by distillation (83~86 °C/0.3 mmHg) to yield 3.72 g (90%) of **3.20a** as a clear liquid: ^{1}H NMR (250 MHz), δ 3.58 (s, 3 H), 3.52 (t, *J* = 6.4 Hz, 2 H), 2.20 (t, J= 7.5 Hz, 2 H), 1.61-1.38 (comp, 4 H), 1.33-1.21 (comp, 2 H), 0.83 (s, 9 H), -0.02 (s, 6 H); ^{13}C NMR (75 MHz) δ 174.1, 62.9, 51.4, 34.0, 32.4, 25.9, 25.4, 24.7, 18.3, -5.4.

A solution of **3.20a** (3.62 mg, 13.9 mmol), 5 N NaOH (2.9 mL, 14.5 mmol) in THF/MeOH/H_2O (10 mL, 2:2:1) was stirred at rt for 7 h. The solution was acidified with 1 N HCl (25 mL) and extracted with EtOAc (3 x 25 mL), dried (Na_2SO_4) and filtered. Solvent was removed under reduced pressure to yield 3.40 g (100%) of **3.21a** as a clear liquid: ^{1}H NMR (300 MHz), δ 10.80-10.20 (br s, 1 H), 3.62 (t, *J* = 6.4 Hz, 2 H), 2.37 (t, *J* = 7.4 Hz, 2 H), 1.72-1.50 (comp, 4 H), 1.48-1.36 (comp, 2 H), 0.90 (s, 9 H), 0.06 (s, 6 H); ^{13}C NMR (75 MHz), δ 180.3, 63.2, 34.3, 32.6, 26.2, 25.6, 24.7, 18.6, -5.1.

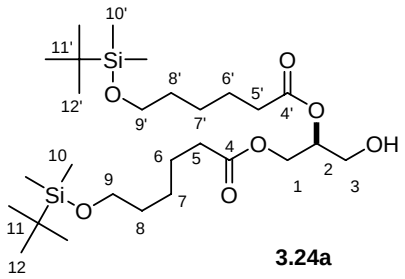


3.23a

1,2-Di-(6'-*tert*-butyldimethylsilyloxyhexanoyl)-3-(*p*-methoxybenzyl)-*sn*-glycerol (3.23a). To a mixture of acid **3.21a** (1.00 g, 4.07 mmol), diol **3.8** (392 mg, 1.85 mmol) and DMAP (50 mg, 0.41 mmol) in CH_2Cl_2 (9 mL) at 0 °C, was added DCC (1.00

g, 4.88 mmol) in CH_2Cl_2 (5 mL). The mixture was stirred at rt for 16 h. The insoluble solid was removed by filtration and washed with CH_2Cl_2. The combined filtrate and washing were concentrated under reduced pressure. The residue was redissolved in ether and filtered. The filtrate was concentrated under reduced pressure, and the residue was purified by chromatography eluting with EtOAc/hexanes (1:8) to yield 0.99 g (80%) of **3.23a** as a clear liquid: ^{1}H NMR (250 MHz) δ 7.16 (d, *J* = 8.6 Hz, 2 H), 6.80 (d, *J* = 8.6 Hz, 2 H), 5.19-5.13 (m, 1 H), 4.42 (d, *J* = 11.7 Hz, 1 H), 4.36 (d, *J* = 11.7 Hz, 1 H), 4.26 (dd, *J* = 11.9, 3.8 Hz, 1 H), 4.10 (dd, *J* = 11.9, 6.4 Hz, 1 H), 3.73 (s, 3 H), 3.55-3.44 (comp, 6 H), 2.29-2.19 (comp, 4 H), 1.68-1.40 (comp, 8 H), 1.35-1.20 (comp, 4 H), 0.82 (s, 18 H), -0.03 (s, 12 H); ^{13}C NMR (62 MHz) δ 173.2, 172.9, 159.3, 129.8, 129.3, 113.8, 72.9, 70.0, 67.8, 62.9, 62.7, 55.2, 34.3, 34.0, 32.4, 25.9, 25.4, 24.7, 24.6, 18.3, -5.3; IR (neat) 2934, 1740, 1613, 1513 cm^{-1}; mass spectrum (CI) *m/z* 669.4190 [$C_{35}H_{65}O_8Si_2$ (M+1) requires 669.4218], 669, 653, 611 (base), 591, 531.

NMR assignments. ^{1}H NMR (250 MHz) δ 7.16 (d, *J* = 8.6 Hz, 2 H, C15-H), 6.80 (d, *J* = 8.6 Hz, 2 H, C16-H), 5.19-5.13 (m, 1 H, C2-H), 4.42 (d, *J* = 11.7 Hz, C13-H), 4.39 (d, *J* = 11.7 Hz, C13-H), 4.26 (dd, *J* = 11.9, 3.8 Hz, 1 H, C1-H), 4.10 (dd, *J* = 11.9, 6.4 Hz, 1 H, C1-H), 3.73 (s, 3 H, C18-H), 3.55-3.44 (comp, 6 H, C3-H & C9-H & C9'-H), 2.29-2.19 (comp, 4 H, C5-H & C5'-H), 1.68-1.40 (comp, 8 H, C8-H & C8'-H & C6-H & C6'-H), 1.35-1.20 (comp, 4 H, C7-H & C7'-H), 0.82 (s, 18 H, C11-H & C11'-H), -0.03 (s, 12 H, C10-H, C10'-H); ^{13}C NMR (62 MHz) δ 173.2 (C4'), 172.9 (C4), 159.3 (C17), 129.8 (C14), 129.3 (C15), 113.8 (C16), 72.9 (C2), 70.0 (C13), 67.8 (C18), 62.9 (C9 & C9'), 62.7 (C1), 55.2 (C3), 34.3 (C5'), 34.0 (C5), 32.4 (C8 & C8'), 25.9 (C11 & C11'), 25.4 (C6 & C6'), 24.7 (C7'), 24.6 (C7), 18.3 (C12 & C12'), -5.3 (C10 & C10').

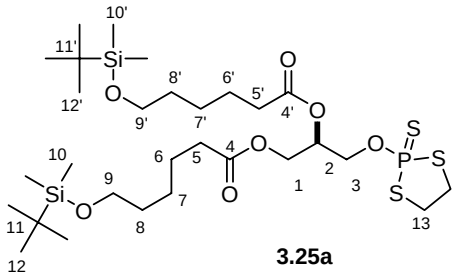


3.24a

1,2-Di-(6'-*tert*-butyldimethylsilyloxyhexanoyl)-*sn*-glycerol (3.24a). To a suspension of DDQ (0.440 g, 1.94 mmol) and CH_2Cl_2/ H_2O (7.2 mL, 10:1), was added PMB ether **3.23a** (0.99 g 1.48 mmol) in CH_2Cl_2(6.5 mL). The solution was stirred at rt for 2 h. The insoluble solid was removed by filtration and washed with CH_2Cl_2 (50 mL). The combined filtrate and washes were washed with $NaHCO_3$ (40 mL), NaCl (40 mL), dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue was purified by chromatography eluting with EtOAc/hexanes (1:5 to 1:4) to yield 660 mg (81%) of **3.24a** as a clear liquid: ^{1}H NMR (300 MHz) δ 5.08-5.03 (m, 1 H), 4.29 (dd, *J* = 11.8, 4.6 Hz, 1 H), 4.20 (dd, *J* = 11.8, 5.6 Hz, 1 H), 3.70-3.69 (comp, 2 H), 3.60-3.55 (comp, 4 H), 2.36-2.28 (comp, 4 H), 2.20-1.96 (br s, 1 H), 1.68-1.45 (comp, 8 H), 1.39-1.30 (comp, 4 H), 0.86 (s, 18 H), 0,02 (s, 12 H); ^{13}C NMR (75 MHz) δ 173.9, 173.5, 72.3, 63.2, 62.2, 61.7, 34.5, 32.7, 32.6, 26.2, 25.6(2), 25.6(0), 25.0, 24.9, 18.6. –5.1; IR (CH_2Cl_2) 3468 (br), 2930, 2858, 1742, 1099, 836, 775 cm^{-1}; mass spectrum (CI) *m/z* 529.3646 [$C_{27}H_{57}O_7Si_2$ (M+1) requires 529.3643] (base), 491, 417, 303, 247, 229.

NMR assignments. ^{1}H NMR (300 MHz) δ 5.08-5.03 (m, 1 H, C2-H), 4.29 (dd, *J* = 11.8, 4.6 Hz, 1 H, C1-H), 4.20 (dd, *J* = 11.8, 5.6 Hz, 1 H, C1-H), 3.70-3.69 (comp, 2 H. C3-H), 3.60-3.55 (comp, 4 H, C9-H & C9'-H), 2.36-2.28 (comp, 4 H, C5-H & C5'-H), 2.20-1.96 (br s, 1 H, OH), 1.68-1.45 (comp, 8 H, C8-H & C8'-H & C6-H & C6'-H), 1.39-1.30 (comp, 4 H, C7-H & C7'-H), 0.86 (s, 18 H, C11-H & C11'-H), 0.02 (s, 12 H,

C10-H & C10'-H); ^{13}C NMR (75 MHz) δ 173.9 (C4'), 173.5 (C4), 72.3 (C2), 63.2 (C9 & C9'), 62.2 (C1), 61.7 (C3), 34.5 (C5'), 32.7 (C5), 32.6 (C8 & C8'), 26.2 (C11 & C11'), 25.62 (C6'), 25.60 (C6), 25.0 (C7'), 24.9 (C7), 18.6 (C12 & C12'), –5.1 (C10 & C10').

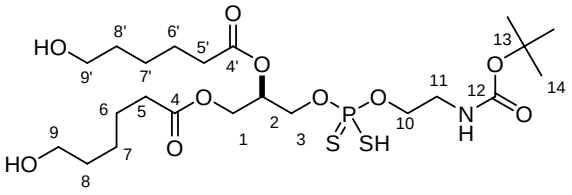


3.25a

2-*O*-1,2-Di-(6'-*tert*-butyldimethylsilyloxyhexanoyl)-*sn*-3-glyceryl-2-thio-1,3,2-dithiophospholane (3.25a). 2-Chloro-1,3,2-dithiophospholane (190 mg, 1.2 mmol) was added dropwise to a solution of **3.24a** (660 mg, 1.20 mmol) and diisopropylethyl-amine (171 mg, 1.32 mmol) in CH_3CN (12 mL) at –38 °C (dry ice/1,2-dichloroethane). The solution was stirred at –38 °C for 2 h. The cold bath was removed and the mixture was stirred for 1 h. A solution of sulfur (136 mg, 4.25 mmol) in CS_2 (4.5 mL) was added in one portion and the solution was stirred vigorously overnight. The mixture was concentrated under reduced pressure and the residue was dissolved in acetone. The insoluble solid was removed by filtration and washed with acetone. The combined filtrate and washing were concentrated under reduced pressure. The residue was purified by chromatography eluting with EtOAc/hexanes (from 1:6 to 1:3) to yield 733 mg (87%) of **3.25a** as a clear liquid: ^{1}H NMR (250 MHz) δ 5.23-5.18 (m, 1 H), 4.27 (dd, *J* = 11.6, 4.5 Hz, 1 H), 4.26-4.14 (m, 2 H), 4.13 (dd, *J* = 11.6, 5.5 Hz, 1 H), 3.68-3.52 (comp, 8 H), 2.33-2.25 (comp, 4 H), 1.66-1.42 (comp, 8 H), 1.37-1.25 (comp, 4 H), 0,83 (s, 18 H), -0.01 (s, 12 H); ^{13}C NMR (75 MHz) δ 173.0, 172.6, 69.1 (d, *J* = 12.6 Hz), 65.3 (d, *J* = 12.6 Hz), 62.8, 61.6, 41.4 (d, *J* = 4.6 Hz), 41.3, 34.0, 33.9, 32.3, 25.8, 25.2, 24.5, 18.2, -

5.4; ^{31}P NMR (121 MHz) δ 124.5; IR (neat) 2931, 1740, 1461 cm^{-1}; mass spectrum (CI) *m/z* 703.2785 [$C_{29}H_{60}O_7Si_2PS_3$ (M+1) requires 703.2777] (base), 687, 645, 531, 457, 256.

NMR assignments. ^{1}H NMR (250 MHz) δ 5.23-5.18 (m, 1 H, C2-H), 4.27 (dd, *J* = 11.6, 4.5 Hz, 1 H, C1-H), 4.26-4.14 (m, 2 H, C3-H), 4.13 (dd, *J* = 11.6, 5.5 Hz, 1 H, C1-H), 3.68-3.52 (comp, 8 H, C9-H & C9'-H & C13-H), 2.33-2.25 (comp, 4 H, C5-H & C5'-H), 1.66-1.42 (comp, 8 H, C6-H & C6'-H & C8-H & C8'-H), 1.37-1.25 (comp, 4 H, C7-H & C7'-H), 0,83 (s, 18 H, C11-H & C11'-H), -0.01 (s, 12 H, C10-H & C10'-H); ^{13}C NMR (75 MHz) δ 173.0 (C4'), 172.6 (C4), 69.1 (d, *J* = 12.6 Hz, C2), 65.3 (d, *J* = 12.6 Hz, C1), 62.8 (C9 & C9'), 61.6 (C3), 41.4 (d, *J* = 4.6 Hz, C13), 34.0 (C5'), 33.9 (C5), 32.3 (C8 & C8'), 25.8 (C11 & C11'), 25.2 (C6 & C6'), 24.5 (C7 & C7'), 18.2 (C12 & C12'), -5.4 (C10 & C10').

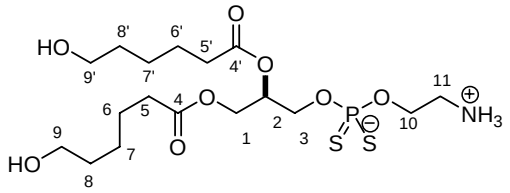


3.28a

***O*-1,2-Di-(6'-hydroxyhexanoyl)-*sn*-3-glyceryl, *O*-(*N*-*tert*-Butoxycarbonyl)-ethanolamine phosphorodithioate (3.28a)**. DBU (47 mg, 0.31 mmol) was added dropwise to a solution of **3.25a** (216 mg, 0.31 mmol) and *N*-Boc-ethanolamine (51 mg, 0.31 mmol) in dry CH_3CN (5 mL). The solution was stirred at rt for 45 min and the solvent was removed under reduced pressure. The residue was purified by column chromatography over silica gel, eluting with acetone/$CHCl_3$ (1:1), to yield 232 mg of **3.27a** as a clear liquid.

The diester **3.27a** (116 mg, 0.11 mmol) thus obtained was dissolved in CH_3CN (2.5 mL) in a plastic bottle, and 2.8 M hydrofluoric acid (0.42 mL) was added. The mixture was stirred at rt for 45 min, and brine (5 mL) was added. The mixture was extracted with $CHCl_3$ (2 x 5 mL). The combined organic phase was dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography eluting with acetone/$CHCl_3$ (3:2) to yield 75 mg (85% after two steps) of **3.28a** as a clear oil: ^{1}H NMR (300 MHz, acetone-d_6) δ 6.27-6.16 (br s, 1 H), 5.29-5.22 (m, 1 H), 4.41 (dd, *J* = 11.8, 3.6 Hz, 1 H), 4.22 (dd, *J* = 11.8, 6.4 Hz, 1 H), 4.11-4.06 (m, 2 H), 4.02-3.95 (m, 2 H), 3.70-3.60 (comp, 2 H), 3.60-3.50 (comp, 4 H), 3.38-3.26 (comp, 2 H), 2.40-2.32 (comp, 4 H), 1.70-1.48 (comp, 8 H), 1.48-1.38 (comp, 13 H); ^{13}C NMR (75 MHz, acetone-d_6) δ 173.5, 173.3, 157.0, 78.8, 71.3 (d, *J* = 9.3 Hz), 64.9, 63.7 (d, *J* = 6.0 Hz), 63.4, 62.2, 41.7, 34.7, 34.5, 33.2, 28.6, 26.0, 25.4. 25.4; ^{31}P NMR (121 MHz, acetone-d_6) δ 118.2; IR (CH_2Cl_2) 3400 (br), 2964, 2936, 1737, 1694, 1171, 1036, 686 cm^{-1}; mass spectrum (CI-1) *m/z* 574.1915 [$C_{22}H_{41}NO_{10}PS_2$ (M-1) requires 574.1910] (base), 473, 433, 291.

NMR assignments. ^{1}H NMR (300 MHz, acetone-d_6) δ 6.27-6.16 (br s, 1 H, NH), 5.29-5.22 (m, 1 H, C2-H), 4.41 (dd, *J* = 11.8, 3.6 Hz, 1 H, C1-H), 4.22 (dd, *J* = 11.8, 6.4 Hz, 1 H, C1-H), 4.11-4.06 (m, 2 H, C3-H), 4.02-3.95 (m, 2 H, C10-H), 3.70-3.60 (comp, 2 H, C11-H), 3.60-3.50 (comp, 4 H, C9-H & C9'-H), 3.38-3.26 (comp, 2 H, OH), 2.40-2.32 (comp, 4 H, C5-H & C5'-H), 1.70-1.48 (comp, 8 H, C6-H & C6'-H & C8-H & C8'-H), 1.48-1.38 (comp, 13 H, C7-H & C7'-H & C14-H); ^{13}C NMR (75 MHz, acetone-d_6) δ 173.5 (C4'), 173.3 (C4), 157.0 (C12), 78.8 (C13), 71.3 (d, *J* = 9.3 Hz, C2), 64.9 (C10), 63.7 (d, *J* = 6.0 Hz, C1), 63.4 (C3), 62.2 (C9 & C9'), 41.7 (C11), 34.7 (C5'), 34.5 (C5), 33.2 (C8 & C8'), 28.6 (C14), 26.0 (C6 & C6'), 25.4 (C7 & C7').

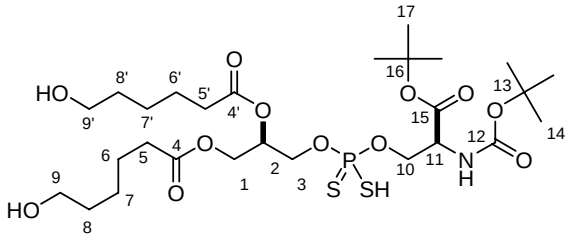


3.4a

***O*-1,2-Di-(6'-hydroxyhexanoyl)-*sn*-3-glyceryl, *O*-(ethanolamine) phosphorodithioate (3.4a).** To a solution of **3.28a** (18 mg, 0.031 mmol) in CH_3CN (2.5 mL) at 0 °C, was added dropwise 1 N $SnCl_4$ in heptane (155 μL, 0.34 mmol). The solution was stirred at rt for 45 min, and the solvent was removed under reduced pressure. The residue was purified by flash chromatography, eluting first with acetone/$CHCl_3$ (3:2), and then with $CHCl_3$/MeOH/H_2O (5:1:0.1), to yield 13 mg (88%) of **3.4a** as a glass: ^{1}H NMR (300 MHz, CD_3OD) δ 5.28-5.20 (m, 1 H), 4.40 (dd, *J* = 11.8, 3.6 Hz, 1 H), 4.23-4.05 (comp, 5 H), 3.55 (t, *J* = 6.6 Hz, 2 H), 3.54 (t, *J* = 6.5 Hz, 2 H), 3.23-3.18 (m, 2 H), 2.40-2.31 (comp, 4 H), 1.70-1.48 (comp, 8 H), 1.48-1.34 (comp, 4 H); ^{13}C NMR (75 MHz, CD_3OD) δ 174.9, 174.6, 71.7, 65.0, 63.7, 63.1, 62.7, 41.3, 35.2, 24.9, 33.2, 16.4(0), 26.3(7), 25.8; ^{31}P NMR (121 MHz, CD_3OD) δ 122.0; IR (MeOH) 3369 (br), 2934, 1732, 1009, 689 cm^{-1}; mass spectrum (FAB) *m/z* 476.1553 [$C_{17}H_{35}NO_8PS_2$ (M+1) requires 476.1542], 277, 242 (base), 185.

NMR assignments. ^{1}H NMR (300 MHz, CD_3OD) δ 5.28-5.20 (m, 1 H, C2-H), 4.40 (dd, *J* = 11.8, 3.6 Hz, 1 H, C1-H), 4.23-4.05 (comp, 5 H, C1-H & C3-H & C10-H), 3.55 (t, *J* = 6.6 Hz, 2 H, C9'-H), 3.54 (t, *J* = 6.5 Hz, 2 H, C9-H), 3.23-3.18 (m, 2 H, C11-H), 2.40-2.31 (comp, 4 H, C5-H & C5'-H), 1.70-1.48 (comp, 8 H, C6-H & C6'-H & C8-H & C8'-H), 1.48-1.34 (comp, 4 H, C7-H & C7'-H); ^{13}C NMR (75 MHz, CD_3OD) δ 174.9 (C4'), 174.6 (C4), 71.7 (C2), 65.0 (C10), 63.7 (C1), 63.1 (C3), 62.7 (C9 & C9'),

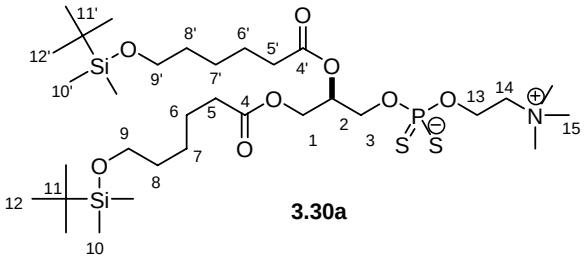
41.3 (C11), 35.2 (C5'), 34.9(C5), 33.2 (C8 & C8'), 16.40 (C6'), 26.37 (C6), 25.8 (C7 & C7').



3.26a

***O*-1,2-Di-(6'-hydroxyhexanoyl)-*sn*-3-glyceryl, *O*-(*N*-*tert*-butoxycarbonyl *L*-serine t-butyl ester) phosphorodithioate (3.26a).** By applying the same method to make **3.27a,** PS **3.26a** was prepared from **3.25a** and *N*-Boc-Ser-*O*-tBu (85%) as a clear liquid: ^{1}H NMR (300 MHz, acetone-d_6) δ 6.43-6.32 (br s, 1 H), 5.30-5.20 (m, 1 H), 4.42 (dd, J = 12.0, 3.3 Hz, 1 H), 4.25-4.20 (comp, 4 H), 4.15-4.05 (comp, 2 H), 3.60-3.52 (comp, 4 H), 2.40-2.32 (comp, 4 H), 1.71-1.38 (comp, 12 H), 1.49 (s, 9 H), 1.44 (s, 9 H); ^{31}P NMR (121 MHz, acetone-d_6) δ 119.3; mass spectrum (CI) *m/z* 676.2595 [$C_{27}H_{51}NO_{12}PS_2$ (M+1) requires 676.2590], (base); (CI-) *m/z* 674 (base), 473, 296.

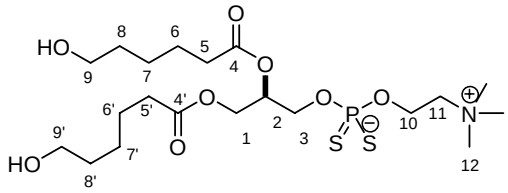
NMR assignments. ^{1}H NMR (300 MHz, acetone-d_6) δ 6.43-6.32 (br s, 1 H, NH), 5.30-5.20 (m, 1 H, C2-H), 4.42 (dd, J = 12.0, 3.3 Hz, 1 H, C1-H), 4.25-4.20 (comp, 4 H, C1-H & C3-H & C11-H), 4.15-4.05 (comp, 2 H, C10-H), 3.60-3.52 (comp, 4 H, C9-H & C9'-H), 2.40-2.32 (comp, 4 H, C5-H & C5'-H), 1.71-1.38 (comp, 12 H, C6-H & C6'-H & C7-H & C7'-H & C8-H & C8'-H), 1.49 (s, 9 H, C17-H), 1.44 (s, 9 H, C14-H).



3.30a

***O*-1,2-Di-(6'-*tert*-butyldimethylsilyloxyhexanoyl)-*sn*-3-glyceryl, *O*-(choline) phosphorodithioate (3.30a).** By applying the same method to make **3.27a,** PC **3.30a** was prepared from **3.25a** and choline tosylate (85%) as a clear liquid: ^{1}H NMR (300 MHz) δ 5.30-5.16 (m, 1 H), 4.66-4.44 (br s, 2 H), 4.34 (dd, *J* = 12.0, 3.1 Hz, 1 H), 4.14-4.06 (comp, 3 H), 3.96-3.86 (br s, 2 H), 3.55 (t, *J* = 6.4 Hz, 4 H), 3.39 (s, 9 H), 2.32-2.23 (comp, 4 H), 1.68-1.39 (comp, 8 H), 1.39-1.22 (comp, 4 H), 0.84 (s, 18 H), -0.02 (s, 12 H); ^{13}C NMR (75 MHz) δ 173.8, 173.3, 70.4 (d, *J* = 9.3 Hz), 66.4, 63.8, 63.2, 59.7, 55.4, 34.6, 34.4, 32.7, 26.2, 25.65, 25.62, 25.0, 24.9, 18.6, -5.0; ^{31}P NMR (121 MHz) δ 117.7; mass spectrum (CI) *m/z* 746.3745[$C_{32}H_{69}NO_8Si_2PS_2$ (M+1) requires 746.3741] (base), 687, 531, 391, 257.

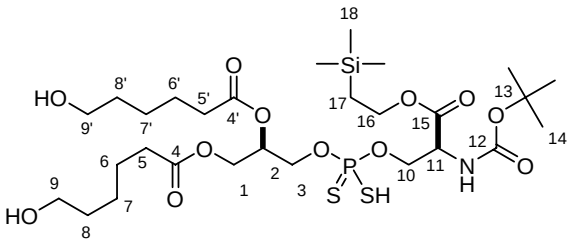
NMR assignments. ^{1}H NMR (300 MHz) δ 5.30-5.16 (m, 1 H, C2-H), 4.66-4.44 (br s, 2 H, C14-H), 4.34 (dd, *J* = 12.0, 3.1 Hz, 1 H, C1-H), 4.14-4.06 (comp, 3 H, C1-H & C3-H), 3.96-3.86 (br s, 2 H, C13-H), 3.55 (t, *J* = 6.4 Hz, 4 H, C9-H & C9'-H), 3.39 (s, 9 H, C15-H), 2.32-2.23 (comp, 4 H, C5-H & C5'-H), 1.68-1.39 (comp, 8 H, C6-H & C6'-H & C8-H & C8'-H), 1.39-1.22 (comp, 4 H, C7-H & C7'-H), 0.84 (s, 18 H, C11-H & C11'-H), -0.02 (s, 12 H, C10-H & C10'-H); ^{13}C NMR (75 MHz) δ 173.8 (C4'), 173.3 (C4), 70.4 (d, *J* = 9.3 Hz, C2), 66.4 (C13), 63.8 (C1), 63.2 (C9 & C9'), 59.7 (C14), 55.4 (C15), 34.6 (C5'), 34.4 (C5), 32.7 (C8 & C8'), 26.2 (C11 & C11'), 25.65 (C6'), 25.62 (C6), 25.0 (C7'), 24.9 (C7), 18.6 (C12 & C12'), -5.0 (C10 & C10'), C3 not observed.



3.3a

***O*-1,2-Di-(6'-hydroxyhexanoyl)-*sn*-3-glyceryl, *O*-(choline) phosphorodithioate (3.3a).** By applying the same method to make **3.28a,** PC **3.3a** was prepared from **3.30a** (95%) as a glass: ^{1}H NMR (300 MHz, CD_3OD) δ 5.32-5.24 (m, 1 H), 4.48-4.38 (comp, 3 H), 4.26-4.08 (comp, 3 H), 3.75-3.70 (comp, 2 H), 3.573 (t, *J* = 6.5 Hz, 2 H), 3.569 (t, *J* = 6.5 Hz, 2 H), 3.29 (s, 9 H), 2.43-2.34 (comp, 4 H), 1.72-1.50 (comp, 8 H), 1.50-1.36 (comp, 4 H); ^{13}C NMR (75 MHz, CD_3OD) δ 174.9, 174.6, 71.8 (d, *J* = 9.2 Hz), 67.2, 64.8 (d, *J* = 5.9 Hz), 63.7, 62.7, 60.6 (d, *J* = 5.0 Hz), 54.9, 35.1, 34.9, 33.3, 26.4, 25.8; ^{31}P NMR (121 MHz, CD_3OD) δ 117.8; mass spectrum (CI) m/z 518.2032 [$C_{20}H_{41}NO_8PS_2$ (M+1) requires 518.2011], 362, 303 (base), 189.

NMR assignments. ^{1}H NMR (300 MHz, CD_3OD) δ 5.32-5.24 (m, 1 H, C2-H), 4.48-4.38 (comp, 3 H, C1-H & C11-H), 4.26-4.08 (comp, 3 H, C1-H & C3-H), 3.75-3.70 (comp, 2 H, C10-H), 3.573 (t, *J* = 6.5 Hz, 2 H. C9'-H), 3.569 (t, *J* = 6.5 Hz, 2 H, C9-H), 3.29 (s, 9 H, C12-H), 2.43-2.34 (comp, 4 H, C5-H & C5'-H), 1.72-1.50 (comp, 8 H, C6-H & C6'-H & C8-H & C8'-H), 1.50-1.36 (comp, 4 H, C7-H & C7'-H); ^{13}C NMR (62 MHz, CD_3OD) δ 174.9 (C4'), 174.6 (C4), 71.8 (d, *J* = 9.2 Hz, C2), 67.2 (C10), 64.8 (d, *J* = 5.9 Hz, C1), 63.7 (C3), 62.7 (C9 & C9'), 60.6 (d, *J* = 5.0 Hz, C11), 54.9 (C12), 35.1 (C5'), 34.9 (C5), 33.3 (C8 & C8'), 26.4 (C6 & C6'), 25.8 (C7 & C7').



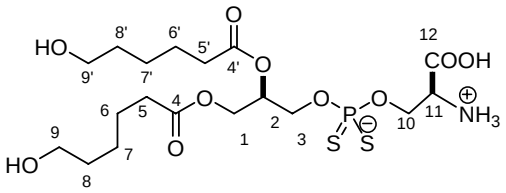
3.39a

***O*-1,2-Di-(6'-hydroxyhexanoyl)-*sn*-3-glyceryl, *O*-(*N*-*tert*-butoxycarbonyl *L*-serine trimethylsilylethyl ester) phosphorodithioate (3.39a)**. DBU (108 mg, 0.71 mmol) was added dropwise to a solution of **3.25a** (500 mg, 0.71 mmol) and trimethylsilyl ethyl *N*-Boc serine ester (**3.37**) (217 mg, 0.71 mmol) in dry CH_3CN (13 mL) at rt. The solution was stirred at rt for 45 min at rt, and the solvent was removed under reduced pressure. The residue was purified by column chromatography eluting with acetone/$CHCl_3$ (1:2) to yield 655 mg of 3.38a as a clear liquid.

The diester **3.38a** (655 mg, 0.596 mmol) thus obtained was dissolved in CH_3CN (20 mL) in a plastic bottle, and 2.8 M HF (3.4 mL) was added. The mixture was stirred at rt for 1 h, and brine (30 mL) was added. The mixture was extracted with $CHCl_3$ (4 x 30 mL). The combined organic phases were dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography eluting with acetone/$CHCl_3$ (3:2) to yield 390 mg (76% after two steps) of 3.39a as a clear oil: ^{1}H NMR (300 MHz, acetone-d_6) δ 6.44 (d, J = 7.4 Hz, 1 H), 5.24-5.17 (m, 1 H), 4.38 (dd, J = 12.0, 3.6 Hz, 1 H), 4.23-4.12 (comp, 6 H), 4.12-4.00 (m, 2 H), 3.60-3.48 (comp, 6 H), 2.40-2.28 (comp, 4 H), 1.70-1.30 (comp, 21 H), 1.10-1.08 (m, 2 H), 0.05 (s, 9 H);^{13}C NMR (75 MHz, acetone-d_6) δ 173.5, 173.3, 171.2, 156.4, 79.2, 71.2 (d, J = 9.3 Hz), 65.2 (d, J = 6.6 Hz), 63.9, 63.7, 63.4, 62.2, 55.5 (d, J = 7.7 Hz), 34.7, 34.5, 33.3, 28.5, 26.1, 26.0, 25.5, 25.4, 17.9, -1.5; ^{31}P NMR (121 MHz, acetone-d_6) δ 119.4; IR (CH_2Cl_2) 3410

(br), 2932, 1731, 1250, 694 cm^{-1}; mass spectrum (CI) *m/z* 718. 2520 [$C_{28}H_{53}NO_{12}PSiS_2$ (M-1) requires 718.2516], 686, 390, 277 (base).

NMR assignments. ^1H NMR (300 MHz, acetone-d_6) δ 6.44 (d, *J* = 7.4 Hz, 1 H, N-H), 5.24-5.17 (m, 1 H, C2-H), 4.38 (dd, *J* = 12.0, 3.6 Hz, 1 H, C1-H), 4.23-4.12 (comp, 6 H, C1-H & C3-H & C10-H & C11-H), 4.12-4.00 (m, 2 H, C16-H), 3.60-3.48 (comp, 6 H, C9-H & C9'-H & OH), 2.40-2.28 (comp, 4 H, C5-H & C5'-H), 1.70-1.30 (comp, 21 H, C6-H & C6'-H & C7-H & C7'-H & C8-H & C8'-H & C14-H), 1.10-1.08 (m, 2 H, C17-H), 0.05 (s, 9 H, C18-H);^{13}C NMR (75 MHz, acetone-d_6) δ 173.5 (C4'), 173.3 (C4), 171.2 (C15), 156.4 (C12), 79.2 (C13), 71.2 (d, *J* = 9.3 Hz, C2), 65.2 (d, *J* = 6.6 Hz, C1), 63.9 (C10), 63.7 (C16), 63.4 (C3), 62.2 (C9 & C9'), 55.5 (d, *J* = 7.7 Hz, C11), 34.7 (C5'), 34.5 (C5), 33.3 (C8'& C8'), 28.5 (C14), 26.1 (C6'), 26.0 (C6), 25.5 (C7'), 25.4 (C7), 17.9 (C17), -1.5 (C18);



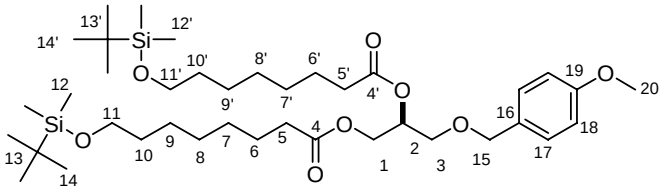
3.5a

***O*-1,2-Di-(6'-hydroxyhexanoyl)-*sn*-3-glyceryl, *O*-(*L*-serine) phosphorodithioate (3.5a)**. A solution of **3.39a** (45 mg, 0.063 mmol) in THF (3 mL) containing 1 N TBAF in THF (187 μL, 0.187 mmol) was stirred for 90 min. The solvent was removed under reduced pressure, and the residue was purified by flash chromatography eluting with acetone/$CHCl_3$ (3:1) to yield 34 mg (88%) of **3.34a** as a clear oil, contaminated with small amount of TBAF: ^{31}P NMR (121 MHz, CD_3OD) δ

119.3; mass spectrum (CI-) *m/z* 618.1793 [$C_{23}H_{41}NO_{12}PS_2$ (M-1) requires 618.1808], 524, 409 (base), 204.

A solution of 1 N $SnCl_4$ in heptane (310 μL, 0.31 mmol) was added to a solution of **3.34a** (34 mg, 0.055 mmol) thus obtained in CH_3CN (5 mL) at 0°C. The mixture was stirred at rt for 1 h and the solvent was removed under reduced pressure. The residue was purified by flash chromatography, eluting first with acetone/$CHCl_3$ (3:1), then with $CHCl_3$/MeOH/H_2O (2:2:0.1), to yield 21.6 mg (75%) of **3.5a** as a glass: ^{1}H NMR (300 MHz, CD_3OD) δ 5.27-5.20 (m, 1 H), 4.52-3.97 (comp, 7 H), 3.55 (t, *J* = 6.4 Hz, 2 H), 3.54 (t, *J* = 6.4 Hz, 2 H), 2.40-2.32 (comp, 4 H), 1.70-1.48 (comp, 8 H), 1.47-1.31 (comp, 4 H); ^{13}C NMR (75 MHz, CD_3OD) δ 175.0, 174.7, 169.4, 71.8 (d, *J* = 9.3 Hz), 64.9 (d, *J* = 6.0 Hz), 64.1 (d, *J* = 6.0 Hz), 63.7, 62.74, 62.73, 54.4 (d, *J* = 8.8 Hz,), 35.1, 34.9, 33.3, 26.42, 26.39, 25.8; ^{31}P NMR (121 MHz, CD_3OD) δ 117.9; mass spectrum (FAB-) *m/z* 519.1357 [$C_{18}H_{37}NO_7P$ (M-) requires 519.1362], 431, 245, 205, 183 (base).

NMR assignments. ^{1}H NMR (300 MHz, CD_3OD) δ 5.27-5.20 (m, 1 H, C2-H), 4.52-3.97 (comp, 7 H, C1-H & C3-H & C10-H & C11-H), 3.55 (t, *J* = 6.4 Hz, 2 H, C9'-H), 3.54 (t, *J* = 6.4 Hz, 2 H, C9-H), 2.40-2.32 (comp, 4 H, C5-H & C5'-H), 1.70-1.48 (comp, 8 H, C6-H & C6'-H & C8-H & C8'-H), 1.47-1.31 (comp, 4 H, C7-H & C7'-H); ^{13}C NMR (75 MHz, CD_3OD) δ 175.0 (C4'), 174.7 (C4), 169.4 (C12), 71.8 (d, *J* = 9.3 Hz, C2), 64.9 (d, *J* = 6.0 Hz, C1), 64.1 (d, *J* = 6.0 Hz, C10), 63.7 (C3), 62.74 (C9'), 62.73 (C9), 54.4 (d, *J* = 8.8 Hz, C11), 35.1 (C5'), 34.9 (C5), 33.3 (C8 & C8'), 26.42 (C6), 26.39 (C6'), 25.8 (C7 & C7').

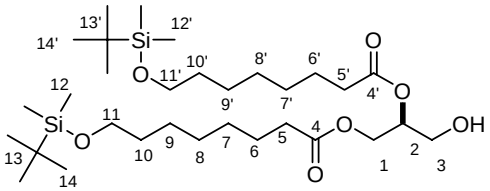


3.23b

1,2-Di-(6'-*tert*-butyldimethylsiloxyoctanoyl)-3-(*p*-methoxybenzyl)-*sn*-glycerol (3.23b). DCC (1.34 g, 6.52 mmol) in CH_2Cl_2 (10 mL) was added to a mixture of acid **3.21b** (1.49 g, 5.43 mmol), diol **3.8** (500 mg, 2.36 mmol) and DMAP (66 mg, 0.54 mmol) in CH_2Cl_2 (8 mL) at 0 °C. The mixture was stirred at rt overnight. The insoluble solid was removed by filtration and washed with ether (50 mL). The combined filtrate and washing were concentrated under reduced pressure. The residue was redissolved in ether (40 mL) and filtered. The filtrate was concentrated under reduced pressure, and the residue was purified by chromatography eluting with EtOAc/hexanes (1:10) to yield 1.30 g (76%) of **3.23b** as a clear liquid: ^{1}H NMR (250 MHz) δ 7.21 (d, *J* = 8.6 Hz, 2 H), 6.84 (d, *J* = 8.6 Hz, 2 H), 5.22-5.15 (m, 1 H), 4.47 (d, *J* = 11.7 Hz, 1 H), 4.41 (d, *J* = 11.7 Hz, 1 H), 4.30 (dd, *J* = 11.9, 3.8 Hz, 1 H), 4.14 (dd, *J* = 11.9, 6.4 Hz, 1 H), 3.77 (s, 3 H), 3.59-3.51 (comp, 6 H), 2.32-2.21 (comp, 4 H), 1.68-1.38 (comp, 8 H), 1.35-1.20 (comp, 12 H), 0.86 (s, 18 H), 0.02 (s, 12 H); ^{13}C NMR (62 MHz) δ 173.2, 172.9, 159.2, 129.6, 129.1, 113.7, 72.8, 69.9, 67.7, 63.0, 62.5, 55.1, 34.1, 33.9, 32.6, 28.9, 25.8, 25.5, 24.7, 24.6, 18.2, -5.3; mass spectrum (CI) *m/z* 725.4832 [$C_{39}H_{73}O_8Si_2$ (M+1) requires 725.4844] (base), 709, 667, 471, 451.

NMR assignments. ^{1}H NMR (250 MHz) δ ^{1}H NMR (250 MHz) δ 7.21 (d, *J* = 8.6 Hz, 2 H, C17-H), 6.84 (d, *J* = 8.6 Hz, 2 H, C18-H), 5.22-5.15 (m, 1 H, C2-H), 4.47 (d, *J* = 11.7 Hz, 1 H, C15-H), 4.41 (d, *J* = 11.7 Hz, 1 H, C15-H), 4.30 (dd, *J* = 11.9, 3.8 Hz, 1 H, C1-H), 4.14 (dd, *J* = 11.9, 6.4 Hz, 1 H, C1-H), 3.77 (s, 3 H C20-H), 3.59-3.51

(comp, 6 H, C3-H & C11-H & C11'-H), 2.32-2.21 (comp, 4 H, C5-H & C5'-H), 1.68-1.38 (comp, 8 H, C10-H & C10'-H & C6-H & C6'-H), 1.35-1.20 (comp, 12 H, C7-H & C7'-H & C8-H & C8'-H & C9-H& C9-H), 0.86 (s, 18 H, C13-H & C13'-H), 0.02 (s, 12 H, C12-H & C12'-H); ^{13}C NMR (62 MHz) δ 173.2 (C4'), 172.9 (C4), 159.2 (C19), 129.6 (C16), 129.1 (C17), 113.7(C18), 72.8 (C2), 69.9 (C15), 67.7 (C20), 63.0 (C11& C11'), 62.5 (C1), 55.1 (C3), 34.1 (C5'), 33.9 (C5), 32.6 (C10 & C10'), 28.9 (C9 & C9' & C6 & C6'), 25.8 (C13 & C13'), 25.5 (C8 & C8'), 24.7(C7'), 24.6(C7), 18.2 (C14 & C14'), -5.3 (C12 & C12').



3.24b

1,2-Di-(6'-*tert*-butyldimethylsilyloxyoctanoyl)-*sn*-glycerol (3.24b). PMB ether **3.23b** (1.30 g, 1.80 mmol) in CH_2Cl_2 (9 mL) was added to a suspension of DDQ (0.53 g, 2.33 mmol) in CH_2Cl_2/H_2O (9 mL, 10:1). The solution was stirred at rt for 2.5 h. The insoluble solid was removed by filtration and washed with CH_2Cl_2 (50 mL). The combined filtrate and washes were washed with $NaHCO_3$ (40 mL), NaCl (40 mL), dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue was purified by chromatography eluting with EtOAc/hexanes (1:5) to yield 1.039 mg (96%) of **3.24b** as a clear liquid: ^{1}H NMR (300 MHz) δ 5.08-5.00 (m, 1 H), 4.29 (dd, *J* = 11.9, 4.4 Hz, 1 H), 4.18 (dd, *J* = 11.9, 5.7 Hz, 1 H), 3.68 (t, *J* = 5.5 Hz, 2 H), 3.58-3.53 (comp, 4 H), 2.33-2.25 (comp, 5 H), 1.68-1.40 (comp, 8 H), 1.38-1.30 (comp, 12 H), 0.85 (s, 18 H), 0.01 (s, 12 H); ^{13}C NMR (75 MHz) δ 173.7, 173.3, 72.1, 63.2, 62.0, 61.4, 34.2, 34.0, 32.7, 30.0,

25.9, 25.6, 24.8, 18.3. –5.3; mass spectrum (CI) *m/z* 605.4273 [$C_{31}H_{65}O_7Si_2$ (M+1) requires 605.4267] (base), 589, 547, 473, 349, 331.

NMR assignments. ^{1}H NMR (300 MHz) δ 5.08-5.00 (m, 1 H, C2-H), 4.29 (dd, *J* = 11.9, 4.4 Hz, 1 H, C1-H), 4.18 (dd, *J* = 11.9, 5.7 Hz, 1 H, C1-H), 3.68 (t, *J* = 5.5 Hz, 2 H, C3-H), 3.58-3.53 (comp, 4 H, C11-H & C11'-H), 2.33-2.25 (comp, 5 H, C5-H & C5'-H & OH), 1.68-1.40 (comp, 8 H, C10-H & C10'-H & C6-H & C6'-H), 1.38-1.30 (comp, 12 H, C7-H & C7'-H & C8-H & C8'-H& C9-H & C9'-H), 0.85 (s, 18 H, C13-H & C13'-H), 0.01 (s, 12 H, C12-H & C12'-H); ^{13}C NMR (75 MHz) δ 173.7 (C4'), 173.3(C4), 72.1(C2), 63.2(C11 & C11'), 62.0(C1), 61.4(C3), 34.2(C5'), 34.0 (C5), 32.7 (C10 & C10'), 30.0 (C9 & C9'& C6 & C6'), 25.9 (C13 & C13'), 25.6 (C8 & C8'), 24.8(C7 & C7'), 18.3 (C14 & C14'), –5.3 (C12 & C12')

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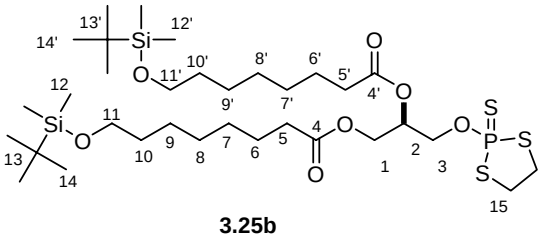
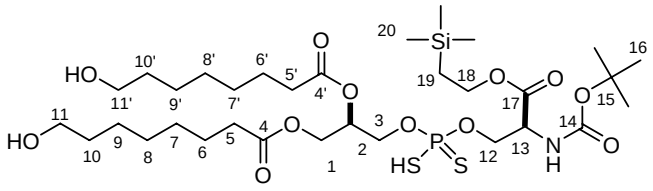


3.25b

2-*O*-1,2-Di-(6'-*tert*-butyldimethylsilyloxyoctanoyl)-*sn*-3-glyceryl-2-thio-1,3,2-dithiophospholane (3.25b). 2-Chloro-1,3,2-dithiophospholane (273 mg, 1.72 mmol) was added dropwise to a solution of **3.24b** (1.039 mg, 1.72 mmol) and *i*Pr_2NEt (246 mg, 1.90 mmol) in CH_3CN (17 mL) at –38 °C (dry ice/1,2-dichloroethane). The solution was stirred at –38 °C for 2 h. The cold bath was removed, and the mixture was stirred for 1 h. A solution of sulfur (191 mg, 5.97 mmol) in CS_2 (6.5 mL) was added in one portion, and the solution was stirred vigorously overnight. The mixture was concentrated under reduced pressure, and the residue was dissolved in acetone. The insoluble solid was

removed by filtration and washed with acetone. The combined filtrate and washing were concentrated under reduced pressure. The residue was purified by chromatography eluting with EtOAc/hexanes (1:7 then 1:4) to yield 1.037 g (80%) of **3.25b** as a clear liquid: ^{1}H NMR (300 MHz) δ 5.25-5.18 (m, 1 H), 4.30-4.09 (comp, 4 H), 3.68-3.52 (comp, 8 H), 2.31-2.24 (comp, 4 H), 1.62-1.38 (comp, 8 H), 1.34-1.20 (comp, 12 H), 0.83 (s, 18 H), -0.02 (s, 12 H); ^{13}C NMR (75 MHz) δ 173.1, 172.6, 69.1 (d, *J* = 9.3 Hz), 65.3 (d, *J* = 8.2 Hz), 63.0, 61.6, 41.4 (d, *J* = 7.2 Hz), 34.0, 33.9, 32.6, 28.9, 28.9, 25.9, 25.5, 24.7, 18.2, -5.4; ^{31}P NMR (121 MHz) δ 124.4; mass spectrum (CI) *m/z* 759.3395 [$C_{33}H_{68}O_7Si_2PS_3$ (M+1) requires 759.3403] (base), 743, 701, 587, 485.

NMR assignments. ^{1}H NMR (250 MHz) δ; ^{13}C NMR (75 MHz) δ. ^{1}H NMR (250 MHz) δ 5.25-5.18 (m, 1 H, C2-H), 4.30-4.09 (comp, 4 H, C1-H & C3-H), 3.68-3.52 (comp, 8 H, C11-H & C11'-H & C15-H), 2.31-2.24 (comp, 4 H, C5-H & C5'-H), 1.62-1.38 (comp, 8 H, C10-H & C10'-H & C6-H & C6'-H), 1.34-1.20 (comp, 12 H, C7-H & C7'-H & C8-H & C8'-H& C9-H & C9'-H), 0.83 (s, 18 H, C13-H & C13'-H), -0.02 (s, 12 H, C12-H & C12'-H); ^{13}C NMR (75 MHz) δ 173.1 (C4'), 172.6 (C4), 69.1 (d, *J* = 9.3 Hz, C2), 65.3 (d, *J* = 8.2 Hz, C1), 63.0 (C11 & C11'), 61.6(C3), 41.4 (d, *J* = 7.2 Hz, C15), 34.0 (C5'), 33.9(C5), 32.6 (C10 & C10'), 28.9 (C9 & C9'& C6 & C6'), 25.9 (C13 & C13'), 25.5(C8 & C8'), 24.7(C7 & C7'),18.2 (C14 & C14'), -5.4 (C12 & C12').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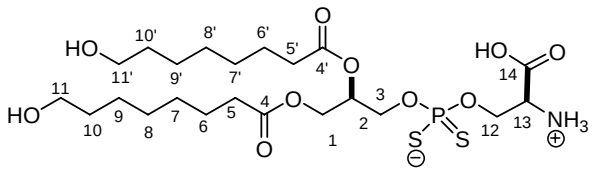
***O*-1,2-Di-(6'-hydroxyhexanoyl)-*sn*-3-glyceryl, *O*-(*N*-*tert*-butoxycarbonyl *L*-serine trimethylsilylethyl ester) phosphorodithioate (3.39b).** By applying the same method to make **3.38aa,** PS **3.38bb** was prepared from **3.25b** (500 mg, 0.66 mmol), trimethylsilylethyl *N*-Boc serine ester (201 mg, 0.66 mmol) and DBU (100 mg, 0.66 mmol) in CH_3CN (12 mL) as a clear liquid (77%, 587 mg); ^{31}P NMR (121 MHz) δ 116.8; mass spectrum (CI) *m/z* 1002.4880 [$C_{44}H_{89}NO_{12}PSi_3S_2$ (M+1) requires 1002.4872] 703, 398 (base).

By applying the same method to make **3.39a,** PS **3.39b** was prepared from **3.38b** (587 mg, 0.508 mmol) and 2.8 M HF (2.9 mL, 8.12 mmol) in CH_3CN (17 mL) as a clear liquid (91%, 359 mg): ^1H NMR (300 MHz, acetone-d_6) δ 6.44 (d, *J* = 7.4 Hz, 1 H), 5.24-5.17 (m, 1 H), 4.40 (dd, *J* = 12.0, 3.3 Hz, 1 H), 4.32-4.10 (comp, 6 H), 4.08-3.98 (comp, 2 H), 3.56-3.42 (comp, 4 H), 2.35-2.27 (comp, 4 H), 1.66-1.22 (comp, 29 H), 1.08-1.00 (m, 2 H), 0.06 (s, 9 H); ^{13}C NMR (75 MHz, acetone-d_6) δ 173.5, 173.2, 171.2, 156.2, 79.2, 71.6 (d, *J* = 4.3 Hz), 65.2 (d, *J* = 6.5 Hz), 63.8, 63.7, 63.5, 62.4, 55.6, (d, *J* = 8.4 Hz), 34.7, 34.5, 33.7, 29.7, 28.6, 26.5, 25.6, 17.9, -1.4; ^{31}P NMR (121 MHz, acetone-d_6) δ 119.6; mass spectrum (CI) *m/z* 774.3112 [$C_{32}H_{61}NO_{12}PSiS_2$ (M+1) requires 774.3142], 411, 340 (base);

NMR assignments. ^1H NMR (300 MHz, acetone-d_6) δ 6.44 (d, *J* = 7.4 Hz, 1 H, N-H), 5.24-5.17 (m, 1 H, C2-H), 4.40 (dd, *J* = 12.0, 3.3 Hz, 1 H, C1-H), 4.32-4.10 (comp, 6 H, C1-H & C3-H & C12-H & C13-H), 4.08-3.98 (comp, 2 H, C18-H), 3.56-3.42 (comp, 4 H, C11-H & C11'-H), 2.35-2.27 (comp, 4 H, C5-H & C5'-H), 1.66-1.22 (comp, 29 H, C6-H & C6'-H & C7-H & C7'-H & C8-H & C8'-H & C9-H & C9'-H & C10-H & C10'-H & C15-H), 1.08-1.00 (m, 2 H, C19-H), 0.06 (s, 9 H, C20-H); ^{13}C NMR (75 MHz, acetone-d_6) δ 173.5(C4'), 173.2(C4), 171.2 (C17), 156.2 (C14), 79.2 (C16), 71.6 (d, *J* = 4.3 Hz, C2), 65.2 (d, *J* = 6.5 Hz, C1), 63.8 (C12), 63.7 (C3), 63.5 (C18), 62.4

(C11 & C11'), 55.6 (d, J = 8.4 Hz, C13), 34.7 (C5'), 34.5 (C5), 33.7(C10 & C10'), 29.7(C9 & C9'), 28.6 (C15), 26.5 (C8 & C8' & C6 & C6'), 25.6(C7 & C7'), 17.9 (C19), -1.4 (C20).



3.5b

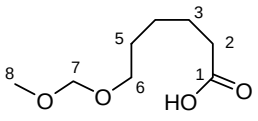
***O*-1,2-Di-(6'-hydroxyoctanoyl)-*sn*-3-glyceryl, *O*-(*L*-serine) phosphorodithioate (3.5b)**.

Method A: A mixture of protected PS **3.29a** (50 mg, 0.047 mmol), ethanedithiol (80 μL, 95 mmol) in TFA (1.5 mL) and thioanisole (1.5 mL) was stirred at rt for 4.5 h. The solvent was removed under reduced pressure. The residue (product, thioanisole and ethanedithiol) was then separated by flash chronography, eluting first with acetone/$CHCl_3$ (3:2) then with MeOH/$CHCl_3$ (1:1). The resulting glass was dissolved in MeOH (2 mL), and stirred at rt for 2 d. The solvent was removed under reduced pressure, and the residue was purified by flash chromatography, eluting first with acetone/$CHCl_3$ (3:2) then with MeOH/$CHCl_3$ (1:1). The resulting glass was then purified by reverse-phase column chromatograph, eluting with CH_3CN/H_2O (9:1) to yield 9 mg (37%) of **3.5b** as a glass.

Method B: By applying the same method to make **3.34a,** PS **3.34b** was prepared from **3.39b** (26 mg, 0.034 mmol), 1 N TBAF in THF (98 μL, 0.098 mmol) in CH_3CN (4.6 mL) as a glass (97%, 22 mg), contaminated with small amount of TBAF; ^{31}P NMR (121 MHz, acetone-d_6) δ 119.4; mass spectrum (CI-) *m/z* 674.2441 [$C_{27}H_{49}NO_{12}PS_2$ (M-1) requires 674.2434], 580, 409 (base), 204.

By applying the same method to make **3.5a,** PS **3.5b** was prepared from **3.34b** (12 mg, 0.018 mmol), 1 N $SnCl_4$ in heptane (103 μL, 0.103 mmol) in CH_3CN (1.7 mL) as a glass (88%, 9 mg): ^1H NMR (300 MHz, CD_3OD) δ; 5.28-5.18 (m, 1 H), 4.50-4.00 (comp, 7 H), 3.68-3.48 (comp, 4 H), 2.40-2.28 (comp, 4 H), 1.70-1.46 (comp, 8 H), 1.46-1.26 (comp, 12 H); ^{13}C NMR (75 MHz, CD_3OD) δ 175.1, 174.8, 169.4, 71.8 (d, *J* = 9.2 Hz), 64.9 (d, *J* = 5.2 Hz), 64.1 (d, *J* = 6.4 Hz), 63.7, 63.0, 54.5 (d, *J* = 8.8 Hz), 35.1, 34.9, 33.6, 30.2, 30.1, 26.8, 25.9; ^{31}P NMR (121 MHz, CD_3OD) δ 118.0; mass spectrum (FAB-) *m/z* 574.1888 [$C_{22}H_{41}NO_{10}PS_2$ (M) requires 574.1910], 487, 275, 244 (base).

NMR assignments. ^1H NMR (300 MHz, CD_3OD) δ 5.28-5.18 (m, 1 H, C2-H), 4.50-4.00 (comp, 7 H, C1-H & C3-H & C12-H & C13-H), 3.68-3.48 (comp, 4 H, C11-H & C11'-H), 2.40-2.28 (comp, 4 H, C5-H & C5'-H), 1.70-1.46 (comp, 8 H, C6-H & C6'-H & C10-H & C10'-H), 1.46-1.26 (comp, 12 H, C7-H & C7'-H & C8-H & C8'-H & C9-H & C9'-H); ^{13}C NMR (75 MHz, CD_3OD) δ 175.1 (C4'), 174.8 (C4), 169.4 (C14), 71.8 (d, *J* = 9.2 Hz, C2), 64.9 (d, *J* = 5.2 Hz, C1), 64.1 (d, *J* = 6.4 Hz, C12), 63.7 (C3), 63.0 (C11 & C11'), 54.5 (d, *J* = 8.8 Hz, C13), 35.1(C5'), 34.9(C5), 33.6 (C10-H & C10'-H), 30.2 (C9-H & C9'-H), 30.1 (C6-H & C6'-H), 26.8 (C8-H & C8'-H), 25.9 (C7-H & C7'-H).

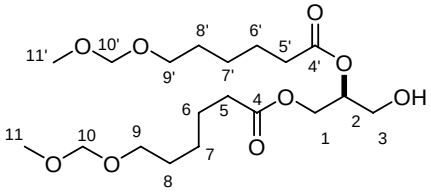


3.41

6-Methoxymethoxyhexanoic acid (3.41). By applying the same basic hydrolysis procedure as to make **3.21a,** acid **3.41** was prepared from the methyl ester (99%) as a clear liquid: ^1H NMR (250 MHz) δ 4.58 (s, 2 H), 3.49 (t, *J* = 6.4 Hz, 2 H), 3.32 (s, 3 H), 2.33 (t, *J* = 7.4 Hz, 2 H), 1.70-1.53 (comp, 4 H), 1.45-1.35 (comp, 2 H); ^{13}C NMR (62

MHz) δ 179.6, 96.3, 67.5, 55.1, 33.9, 29.3, 25.7, 24.4; IR (neat) 3100 (br), 2940, 1709, 1411 cm^{-1}; mass spectrum (CI) m/z 177.1129 [$C_8H_{17}O_4$ (M+1) requires 177.1127], 159, 145, 115 (base).

NMR assignments. ^{1}H NMR (250 MHz) δ 4.58 (s, 2 H, C7-H), 3.49 (t, *J* = 6.4 Hz, 2 H, C5-H), 3.32 (s, 3 H, C8-H), 2.33 (t, *J* = 7.4 Hz, 2 H, C2-H), 1.70-1.53 (comp, 4 H, C3-H & C5-H), 1.45-1.35 (comp, 2 H, C4-H); ^{13}C NMR (62 MHz) δ 179.6 (C1), 96.3 (C7), 67.5 (C8), 55.1 (C6), 33.9 (C2), 29.3 (C5), 25.7 (C3), 24.4 (C4).



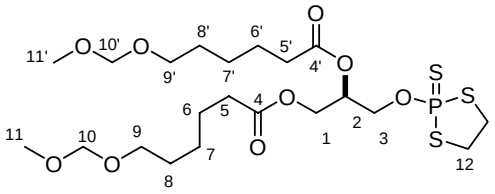
3.43

1,2-Di-(6'-methoxymethoxyhexanoyl)-*sn*-glycerylol (3.43). By applying the same DCC coupling procedure as to make **3.23a,** diester **3.42** was prepared from **3.8** as a clear liquid. ^{1}H NMR (300 MHz) δ 7.19 (d, *J* = 8.6 Hz, 2 H), 6.83 (d, *J* = 8.6 Hz, 2 H), 5.17 (m, 1 H), 4.56 (s, 4 H), 4.45 (d, *J* = 11.7 Hz, 1 H), 4.39 (d, *J* = 11.7 Hz, 1 H), 4.29 (dd, *J* = 11.9, 3.8 Hz, 1 H), 4.12 (dd, *J* = 11.9, 76.4 Hz, 1 H), 3.76 (s, 3 H), 3.52-3.44 (comp, 6 H), 3.31 (s, 6 H), 2.33-2.22 (comp, 4 H), 1.67-1.50 (comp, 8 H), 1.42-1.31 (comp, 4 H); ^{13}C NMR (62 MHz) δ 175.0, 172.7, 159.2, 129.6, 129.1, 113.6, 72.8, 69.9, 67.7, 67.3, 62.6, 55.1, 54.9, 34.0, 33.8, 29.2, 25.6, 25.5, 24.5, 24.4; IR ($CHCl_3$) 2934, 1739, 1612, 1513, 1464 cm^{-1}; mass spectrum (CI) m/z 527.2857 [$C_{27}H_{43}O_{10}$ (M-1) requires 527.2856], 383, 351(base).

By applying the same DDQ cleavage procedure as to make **3.24a,** alcohol **3.43** was prepared from **3.42** (83% after two steps) as a clear liquid: ^{1}H NMR (300 MHz) δ

5.05 (m, 1 H), 4.58 (s, 4 H), 4.29 (dd, *J* = 11.8, 7.2 Hz, 1 H), 4.17 (dd, *J* = 11.8, 6.1 Hz, 1 H), 3.70-3.68 (comp, 2 H), 3.51-3.46 (comp, 4 H), 3.32 (s, 6 H), 2.37-2.15 (comp, 5 H), 1.70-1.53 (comp, 8 H), 1.43-1.34 (comp, 4 H); ^{13}C NMR (75 MHz) δ 173.8, 173.4, 96.6, 72.4, 67.7(1), 67.6(9), 62.3, 61.7, 55.4, 34.4, 34.2, 29.6, 29.5, 26.0, 25.9, 24.9; IR ($CHCl_3$) 3465, 2938, 1738 cm^{-1}; mass spectrum (CI) m/z 409.2430 [$C_{19}H_{37}O_9$ (M+1) requires 409.2438], 377, 345 (base), 333, 315, 231, 201.

NMR assignments. ^{1}H NMR (300 MHz) δ 5.05 (m, 1 H, C2-H), 4.58 (s, 4 H, C10-H & C10'-H), 4.29 (dd, *J* = 11.8, 7.2 Hz, 1 H, C1-H), 4.17 (dd, *J* = 11.8, 6.1 Hz, 1 H, C1-H), 3.70-3.68 (comp, 2 H, C3-H), 3.51-3.46 (comp, 4 H, C9-H & C9'-H), 3.32 (s, 6 H, C11-H), 2.37-2.15 (comp, 5 H, OH & C5-H & C5'-H), 1.70-1.53 (comp, 8 H, C6-H & C6'-H & C8-H & C8'-H), 1.43-1.34 (comp, 4 H, C7-H & C7'-H); ^{13}C NMR (75 MHz) δ 173.8 (C4'), 173.4 (C4), 96.6 (C10 & C10'), 72.4 (C2), 67.7 (C11 & C11'), 62.3 (C1), 61.7 (C3), 55.4 (C9 & C9'), 34.4 (C5'), 34.2 (C5), 29.6(C8'), 29.5 (C8), 26.0 (C6'), 25.9 (C6), 24.9 (C7 & C7').

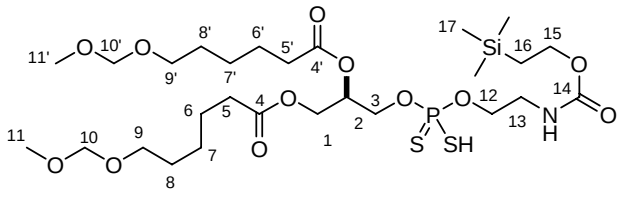


3.44

2-*O*-1,2-Di-(6'-methoxymethoxyhexanoyl)-*sn*-3-glyceryl-2-thio-1,3,2-dithiophospholane (3.44). By applying the same method to make **3.25a,** dithiophospholane **3.44** was prepared from **3.43** (65%) as a clear liquid: ^{1}H NMR (300 MHz) δ 5.27-5.20 (m, 1 H), 4.58 (s, 4 H), 4.32-4.13 (comp, 4 H), 3.69-3.54 (comp, 4 H), 3.51-3.46 (comp, 4 H), 3.32 (s, 6 H), 2.36-2.29 (comp, 4 H), 1.69-1.53 (comp, 8 H), 1.43-

1.34 (comp, 4 H); ^{13}C NMR (75 MHz) δ 173.3, 172.9, 96.7, 69.5 (d, *J* = 8.8 Hz), 67.7, 65.7 (d, *J* = 8.3 Hz), 62.0, 55.4, 41.8 (d, *J* = 5.5 Hz), 34.4, 34.2, 29.6, 26.0, 26.0, 24.9; ^{31}P NMR (121 MHz) δ 124.6; IR (neat) 2937, 1740, 1458 cm^{-1}; mass spectrum (CI-) m/z 561.1395 [$C_{21}H_{38}O_9PS_3$ (M-1) requires 527.2856], m/z 561, 534 (base), 216

NMR assignments. ^{1}H NMR (300 MHz) δ 5.27-5.20 (m, 1 H, C2-H), 4.58 (s, 4 H, C10-H & C10'-H), 4.32-4.13 (comp, 4 H, C1-H & C3-H), 3.69-3.34 (comp, 4 H, C12-H), 3.51-3.46 (comp, 4 H, C9-H & C9'-H), 3.32 (s, 6 H, C11-H & C11'-H), 2.36-2.29 (comp, 4 H, C5-H & C5'-H), 1.69-1.53 (comp, 8 H, C6-H & C6'-H & C8-H & C8'-H), 1.43-1.34 (comp, 4 H, C7-H & C7'-H); ^{13}C NMR (75 MHz) δ 173.3 (C4'), 172.9 (C4), 96.7 (C10 & C10'), 69.5 (d, *J* = 8.8 Hz, C2), 67.7 (C11), 65.7 (d, *J* = 8.3 Hz, C1), 62.0 (C3), 55.4 (C9), 41.8 (d, *J* = 5.5 Hz, C12), 34.4 (C4'), 34.2 (C4), 29.6 (C8 & C8'), 26.0 (C6 & C6'), 24.9 (C7 & C7').

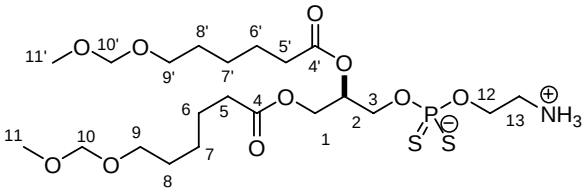


3.45b

***O*-1,2-Di-(6'-methoxymethoxyhexanoyl)-*sn*-3-glyceryl, *O*-(*N*-trimethylsilylethoxycarbonyl ethanolamine) phosphorodithioate (3.45b).** DBU (15.6 mg, 0.10 mmol) was added dropwise to a solution of **3.44** (55 mg, 0.098 mmol) and *N*-Teoc ethanolamine (21 mg, 0.10 mmol) in dry CH_3CN (2.5 mL) at rt. The solution was stirred at rt for 15 min, and the solvent was removed under reduced pressure. The residue was purified by flash chromatography eluting first with EtOAc then with EtOAc/MeOH (10:1) to yield 31 mg (45%) of **3.45b** as a clear liquid: ^{1}H NMR (250 MHz) δ 5.55-5.42

(br s, 1 H), 5.35-5.25 (m, 1 H), 4.59 (s, 4 H), 4.33 (dd, *J* = 12.3, 3.4 Hz, 1 H), 4.25-3.90 (comp, 7 H), 3.52-3.35 (comp, 6 H), 3.32 (s, 6 H), 2.40-2.25 (comp, 4 H), 1.70-1.50 (comp, 8 H), 1.46-1.20 (comp, 4 H), 1.20-0.88 (m, 2 H), 0.00 (s, 9 H); ^{13}C NMR (75 MHz, CD_3OD) δ 173.9, 173.8, 158.1, 96.3, 70.5, 67.5, 66.0, 64.0, 63.6, 62.6, 55.1, 41.3, 34.3, 34.0, 29.3, 25.7, 25.6, 24.5, 17.6, -1.5; mass spectrum (CI) *m/z* 706.2515 [$C_{27}H_{53}NO_{12}PSiS_2$ (M-1) requires 706.2516], 240, 211 (base).

NMR assignments. ^{1}H NMR (250 MHz) δ 5.55-5.42 (br s, 1 H, N-H), 5.35-5.25 (m, 1 H, C2-H), 4.59 (s, 4 H, C10-H & C10'-H), 4.33 (dd, *J* = 12.3, 3.4 Hz, 1 H, C1-H), 4.25-3.90 (comp, 7 H, C1-H & C3-H & C12-H & C14-H), 3.52-3.35 (comp, 6 H, C9-H & C9'-H & C13-H), 3.32 (s, 6 H, C11-H & C11'-H), 2.40-2.25 (comp, 4 H, C5-H & C5'-H), 1.70-1.50 (comp, 8 H, C6-H & C6'-H & C8-H & C8'-H), 1.46-1.20 (comp, 4 H, C7-H & C7'-H), 1.20-0.88 (m, 2 H, C16-H), 0.00 (s, 9 H, C7-H); ^{13}C NMR (75 MHz, CD_3OD) δ 173.9 (C4'), 173.8 (C4), 158.1 (C14), 96.3 (C10 & C10'), 70.5, 67.5 (C9 & C9'), 66.0 (C1), 64.0 (C15), 63.6 (C12), 62.6 (C3), 55.1 (C11 & C11'), 41.3 (C13), 34.3 (C5'), 34.0 (C5), 29.3 (C8 & C8'), 25.7 (C6'), 25.6 (C6), 24.5 (C7 & C7'), 17.6 (C16), -1.5 (C17)

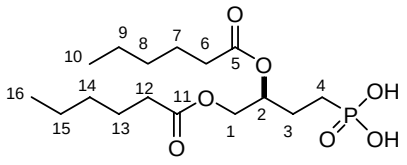


3.46

***O*-1,2-Di-(6'-methoxymethoxyhexanoyl)-*sn*-3-glyceryl, *O*-(ethanolamine) phosphorodithioate (3.46).** A solution of **3.45b** (13 mg, 0.018 mmol) in CH_3CN (2.0 mL) containing 1 N TBAF in THF (60 μL, 0.06 mmol) was stirred for 1 d. The solvent

was removed under reduced pressure and the residue was purified by flash chromatography eluting with acetone/$CHCl_3$ (1:2 then 1:1) to yield 4 mg (39%) of **3.46** as a pale yellow glass:^{1}H NMR (300 MHz, CD_3OD) δ 5.32-5.22 (m, 1 H), 4.59 (s, 4 H), 4.44-4.08 (comp, 6 H), 3.51 (t, *J* = 6.5 Hz, 2 H), 3.50 (t, *J* = 6.5 Hz, 2 H), 3.48-3.40 (m, 2 H), 3.34 (s, 6 H), 2.40-2.38 (comp, 4 H), 1.70-1.50 (comp, 8 H), 1.48-1.32 (comp, 4 H); ^{13}C NMR (75 MHz, CD_3OD) δ 173.6, 173.3, 96.4, 70.2 (d, *J* = 8.7 Hz), 67.62, 67.57, 64.2 (d, *J* = 5.9 Hz), 62.6, 62.2 (d, *J*= 5.2 Hz), 55.21, 55.18, 41.2, 34.2, 34.1, 29.7, 29.4, 25.7, 25.73, 25.66, 24.7, 24.6; ^{31}P NMR (121 MHz, CD_3OD) δ 116.7; mass spectrum (CI) *m/z* 564.2076 [$C_{21}H_{43}NO_{10}PS_2$ (M+1) requires 564.2066]; mass spectrum (CI-) *m/z* 562, 517, 443 (base).

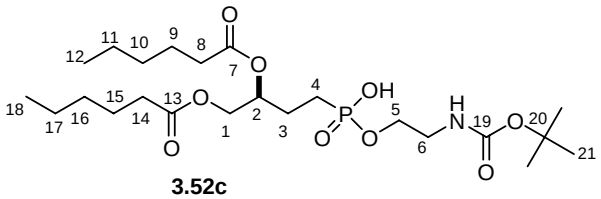
NMR assignments. ^{1}H NMR (300 MHz, CD_3OD) δ 5.32-5.22 (m, 1 H, C2-H), 4.59 (s, 4 H, C10-H & C10'-H), 4.44-4.08 (comp, 6 H, C1-H & C3- H & C12-H), 3.51 (t, *J* = 6.5 Hz, 2 H, C9'-H), 3.50 (t, *J* = 6.5 Hz, 2 H, C9-H), 3.48-3.40 (m, 2 H, C13-H), 3.34 (s, 6 H, C11-H & C11'-H), 2.40-2.38 (comp, 4 H, C5-H & C5'-H), 1.70-1.50 (comp, 8 H, C6-H & C6'-H & C8-H & C8'-H), 1.48-1.32 (comp, 4 H, C7-H & C7'-H); ^{13}C NMR (75 MHz, CD_3OD) δ 173.6 (C4'), 173.3 (C4), 96.4 (C10 & C10'), 70.2 (d, *J* = 8.7 Hz, C2), 67.62 (C9'), 67.57 (C9), 64.2 (d, *J* = 5.9 Hz, C1), 62.6 (C12), 62.2 (d, *J* = 5.2 Hz, C3), 55.21 (C11'), 55.18 (C11), 41.2 (C13), 34.2 (C5'), 34.1 (C5), 29.7 (C8'), 29.4 (C8), 25.73 (C6'), 25.66 (C6), 24.7 (C7'), 24.6 (C7).



1.58c

3-(S)-Bis(*n*-hexanoyloxy)butyl]phosphonic acid (1.58c). To a solution of the methyl phsphonate **3.51c** (186 mg, 0.44 mmol) in dry CH_2Cl_2 (0.5 mL), was added bromotrimethylsilane (152 μL, 1.15 mmol) at rt over 30 min. The solution was stirred for 6 h at rt. The solvent was removed under reduced pressure, and THF/H_2O (1 mL, 1:9) was added. The mixture was refluxed for 1 h. Solvent was removed under reduced pressure and the residue oil was dissolved in $CHCl_3$ (5 mL), dried (Na_2SO_4), filtered and evaporated to yield 160 mg of **1.58c** as pale yellow liquid, which is sufficiently pure by NMR: ^{1}H NMR (300 MHz) δ 11.00-9.40 (br, s, 2 H), 5.10-5.00 (br, s, 1 H), 4.28 (d, *J* = 10.2 Hz, 1 H), 4.18-3.92 (br, s, 1 H), 2.38-2.24 (comp, 4 H), 2.00-1.70 (br, s, 3 H), 1.78-1.52 (comp, 5 H), 1.40-1.20 (br, s, 8 H), 0.96-0.80 (m, 6 H); ^{13}C NMR (75 MHz) δ 173.9, 173.8, 71.3, 34.4 (d, *J* = 17.5 Hz), 31.4, 29.9, 24.7 (d, *J* = 7.1 Hz), 23.5 (d, *J* = 77.6 Hz), 22.5, 14.1; ^{31}P NMR (121 MHz) δ 35.0; IR ($CHCl_3$) 3580, 2958, 1708, 1460, 1414 cm^{-1}; mass spectrum (CI) *m/z* 367.1882 [$C_{18}H_{37}NO_7P$ (M+1) requires 367.1886], 233(base).

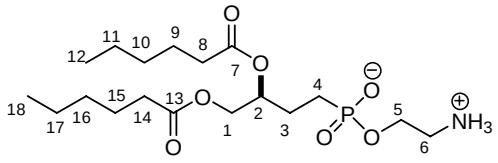
NMR assignments. ^{1}H NMR (300 MHz) δ 11.00-9.40 (br, s, 2 H, O-H), 5.10-5.00 (br, s, 1 H, C2-H), 4.28 (d, *J* = 10.2 Hz, 1 H, C1-H), 4.18-3.92 (br, s, 1 H, C1-H), 2.38-2.24 (comp, 4 H, C6-H & C12-H), 2.00-1.70 (br, s, 3 H, C13-H & C7-H), 1.78-1.52 (comp, 5 H, C3-H & C4-H & C13-H), 1.40-1.20 (br, s, 8 H, C8-H & C9-H & C14-H & C15-H), 0.96-0.80 (m, 6 H, C10-H & C16-H); ^{13}C NMR (75 MHz) δ 173.9 (C5), 173.8 (C11), 71.3 (C2), 64.6 (C1), 34.5 (C6), 34.2 (C12), 31.4 (C7 & C13), 29.9 (C3), 24.7 (C8), 24.0 (C14), 23.5 (d, *J* = 77.6 Hz, C4), 22.5 (C9 & C15), 14.1(C10 & C16).



3.52c

***N*-*tert*-Butoxycarbonyl ethanolamine 3-(*S*)-Bis(*n*-hexanoyloxy)butyl]-phosphonate (3.52c).** To a solution of phosphonic acid **1.58c** (90 mg, 0.25 mmol) and *N*-Boc- ethanolamine (183 mg, 1.14 mmol) in dry pyridine (6.6 mL) at 60 °C, was added trichloroacetonitrile (1.05 mL, 10.5 mmol). The reaction mixture was stirred at 62-75 °C (oil bath) for 46 h. The Pyridine was removed by distillation under vacuum. The dark orange residue was dissolved in CH_2Cl_2 (5 mL) and treated with activated carbon. The mixture was filtered through celite, concentrated, and the residue was purified by chromatography, eluting with $CHCl_3$/MeOH (2:1), to yield 62 mg (49%) of **3.52c** as a yellow oil: ^{1}H NMR (300 MHz) δ 5.10-5.00 (br, m, 1 H), 4.22 (d, *J* = 10 Hz, 1 H), 3.90 (dd, *J* = 11.7, 7.4 Hz, 1 H), 3.85-3.75 (br, s, 2 H), 3.30-3.05 (br, comp, 2 H), 2.25-2.17 (comp, 4 H), 1.78-1.62 (br, s, 2 H), 1.60-1.42 (comp, 6 H), 1.36 (s, 9 H), 1.30-1.19 (comp, 8 H), 0.86-0.78 (comp, 6 H); ^{13}C NMR (75 MHz) δ 173.8, 156.8, 79.4, 72.1 (d, *J* = 15.3 Hz), 65.0, 63.9, 41.7, 34.5, 34.2, 31.5, 30.5, 28.6, 25.2, 24.9, 24.7, 22.6, 14.15, 14.13; ^{31}P NMR (121 MHz) δ 25.4; IR ($CHCl_3$) 3580, 3018, 1734, 1216 cm^{-1}; mass spectrum (CI) *m/z* 510.2828 [$C_{23}H_{45}NO_9P$ (M+1) requires 510.2832] (base), 480, 462, 433, 419 (base).

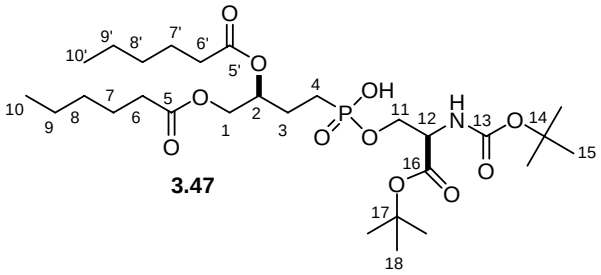
NMR assignments. ^{1}H NMR (300 MHz) δ 5.10-5.00 (br, m, 1 H, C2-H), 4.22 (d, *J* = 10 Hz, 1 H, C1-H), 3.90 (dd, *J* = 11.7, 7.4 Hz, 1 H, C1-H), 3.85-3.75 (br, s, 2 H, C5-H), 3.30-3.05 (br, comp, 2 H, C6-H), 2.25-2.17 (comp, 4 H, C8-H, C14-H), 1.78-1.62 (br, s, 2 H, C4-H), 1.60-1.42 (comp, 6 H, C9-H & C15-H & C3-H), 1.36 (s, 9 H, C21-H), 1.30-1.18 (comp, 8 H, C10-H & C11-H & C16-H & C17-H), 0.86-0.78 (comp, 6 H, C12-H & C-18 H); ^{13}C NMR (75 MHz) δ 173.8 (C7 & C13), 156.8 (C19), 79.4 (C20), 72.1 (d, *J* = 15.3 Hz, C2), 65.0 (C1), 63.9 (C5), 41.7 (C6), 34.5 (C8), 34.2(C14), 31.5 (C21), 30.5 (C9 & C15), 28.6(C3), 25.2 (C3), 24.9 (C10), 24.7 (C16), 22.6(C11 & C17), 14.15 (C12), 14.13 (C18).



3.53c

Ethanolamine 3-(*S*)-Bis(*n*-hexanoyloxy)butyl]phosphonate (3.53c). *N*-Boc protected PE **3.52c** (26 mg, 0.051 mmol) was dissolved in dry CH_2Cl_2 (5 mL) containing TFA (0.5 mL), and the solution was stirred at rt for 35 min, cyclohexane (9 mL) was added to the pink solution, solvent was removed under reduced pressure and the residue was purified by chromatography eluting with $CHCl_3$/MeOH (2:3) to yield 11 mg (55%) of **3.53c** as a glass: ^{1}H NMR (300 MHz) δ 7.20-5.20 (br, s, 3 H), 5.11-5.00 (m, 1 H), 4.20 (dd, *J* = 11.8, 2.8 Hz, 1 H), 4.06-3.90 (comp, 3 H), 2.98 (s, 2 H), 2.26-2.19 (comp, 4 H), 1.77-1.69 (m, 2 H), 1.60-1.48 (comp, 6 H), 1.23 (comp, 8 H), 0.85-0.80 (m, 6 H); ^{13}C NMR (75 MHz) δ 173.5, 173.4, 71.5 (d, *J* = 14.8 Hz), 64.6, 61.3, 40.5, 34.3, 34.0, 31.2, 25.3, 24.6, 24.5, 22.4 (d, *J* = 138 Hz), 22.3, 13.9; ^{31}P NMR (121 MHz) δ 27.2; mass spectrum (CI) *m/z* 410.2310 [$C_{18}H_{37}NO_7P$ (M+1) requires 410.2308] (base), 392, 294 251, 170.

NMR assignments. ^{1}H NMR (300 MHz) δ 7.20-6.20 (br, s, 3 H, N-H), 5.11-5.00 (m, 1 H, C2-H), 4.20 (dd, *J* = 11.8, 2.8 Hz, 1 H, C1-H), 4.06-3.90 (comp, 3 H, C1-H & C-5 H), 2.98 (s, 2 H, C6-H), 2.26-2.19 (comp, 4 H, C8-H, C14-H), 1.77-1.69 (m, 2 H, C4-H), 1.60-1.48 (comp, 6 H, C9-H & C15-H & C3-H), 1.23 (comp, 8 H, C10-H & C11-H & C16-H & C17-H), 0.85-0.80 (m, 6 H, C12-H & C-18 H); ^{13}C NMR (75 MHz) δ 173.5 (C7), 173.4 (C13), 71.5 (d, *J* = 14.8 Hz, C2), 64.6 (C1), 61.3 (C5), 40.5 (C6), 34.3 (C8), 34.0 (C14), 31.2 (C9 & C15), 25.3 (C3), 24.6(C10), 24.5 (C16), 22.4 (d, *J* = 138 Hz, C4), 22.3 (C11 & C17), 13.9 (C12 & C18).

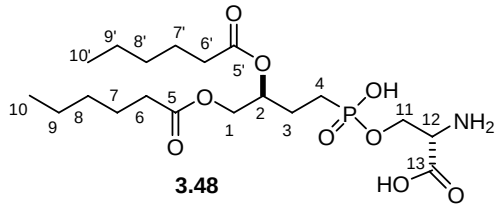


3.47

3(*S*), 4-Dihexanoyloxybutyl (*N*-*tert*-butoxycarbonyl *L*-serine *t*-butyl ester) 1-phosphonate (3.47). Trichloroacetonitrile (1.62 mL, 16.2 mmol) was added to a solution of phosphonic acid **1.68c** (136 mg, 0.372 mmol) and *N*-Boc-Ser-O-tBu (292 mg, 1.16 mmol) in dry pyridine (10 mL) at 60 °C, The reaction mixture was stirred at 60-70 °C (bath temperature) for 22 h. The pyridine was removed by distillation under vacuum. The dark orange residue was dissolved in CH_2Cl_2 (5 mL) and treated with activated carbon. The mixture was filtered through celite, concentrated, and the residue was purified by flash chromatography, eluting with $CHCl_3$/MeOH (4:1) to yield 173 mg (76%) of **3.47**as a yellow glass:^{1}H NMR (250 MHz) δ 5.15-5.02 (br s, 1 H), 4.35-3.90 (comp, 6 H), 2.27-2.20 (comp, 4 H), 1.85-1.68 (br s, 2 H), 1.68-1.45 (comp, 6 H), 1.45-1.18 (comp, 26 H), 0.87-0.81 (comp, 6 H); ^{13}C NMR (75 MHz) δ 173.4, 155.9, 82,4, 78.6, 71.9, 64.8, 56.4, 55.1, 34.3, 34.0, 31.2, 28.4, 28.2, 28.0, 24.9, 24.6, 24.4, 22.3(d, J = 155.6 Hz), 13.9; ^{31}P NMR (121 MHz) 25.8; mass spectrum (CI) m/z 610.3356 [$C_{28}H_{53}NO_{11}P$ (M+1) requires 610.3356], 419, 262, 244 (base).

NMR assignments. ^{1}H NMR (250 MHz) δ 5.15-5.02 (br s, 1 H, C2-H), 4.35-3.90 (comp, 6 H, C1-H & C11-H & C12-H & NH), 2.27-2.20 (comp, 4 H, C6-H & C6'-H), 1.85-1.68 (br s, 2 H, C3-H), 1.68-1.45 (comp, 6 H, C4-H & C7-H & C7'-H), 1.45-1.18 (comp, 26 H, C15-H & C18-H & C8-H & C8-H & C9-H & C9'-H), 0.87-0.81 (comp, 6 H, C10-H & C10-H); ^{13}C NMR (75 MHz) δ 173.4 (C5 & C5'), 155.9 (C13), 82.4 (C17),

78.6 (C14), 71.9 (C2), 64.8 (C1), 56.4 (C11), 55.1 (C12), 34.3 (C6'), 34.0 (C6), 31.2 (C18), 28.4 (C7'), 28.2 (C7), 28.0 (C15), 24.9 (C3), 24.6 (C8 & C8'), 24.4 (C9 & C9'), 22.3(d, J = 155.6 Hz, C4), 13.9 (C10 & C10'), C16 not observed.

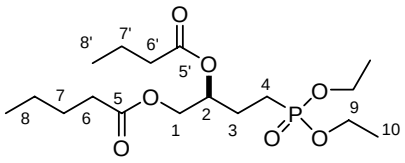


3.48

3(*S*), 4-Dihexanoyloxybutyl-1-phosphonyl *L*-serine (3.48). Protected PS **3.47** (96 mg, 0.16 mmol) was dissolved in dry CH_2Cl_2 (4.0 mL) containing TFA (4.0 mL) at 0 °C, and the solution was stirred at rt for 4.5 h. The solvent was removed under reduced pressure, and the residue was purified by chromatography eluting with $CHCl_3$/MeOH (from 3:1 to 4:7) to yield 63 mg (88%) of **3.48** as a glass:^{1}H NMR (300 MHz, CD_3OD/TFA-*d*) δ 5.14-5.05 (m, 1 H), 4.40-4.32 (comp, 4 H), 4.03 (dd, J = 11.8, 6.4 Hz, 1 H), 2.36-2.26 (comp, 4 H), 1.94-1.52 (comp, 8 H), 1.40-1.24 (comp, 8 H), 0.97-0.85 (comp, 6 H); ^{13}C NMR (75 MHz, CD_3OD/TFA-*d*) δ 175.03, 174.97, 169.2, 72.9 (d, J = 17.5 Hz), 65.5, 63.2 (d, J = 6.0 Hz), 54.6 (d, J = 4.9 Hz), 35.1, 34.9, 32.4, 25.7, 25.6, 25.5 (d, J = 0.5 Hz), 23.4, 23.1 (d, J = 139.3), 14.3; ^{31}P NMR (121 MHz) δ 35.1; mass spectrum (CI-) m/z 452.2039 [$C_{19}H_{35}NO_9P$ (M-1) requires 452.2049] (base), 365, 305.

NMR assignments. ^{1}H NMR (300 MHz, CD_3OD/TFA-*d*) δ 5.14-5.05 (m, 1 H, C2-H), 4.40-4.32 (comp, 4 H, C1-H & C12-H & C11-H), 4.03 (dd, J = 11.8, 6.4 Hz, 1 H. C1-H), 2.36-2.26 (comp, 4 H, C6-H & C6'-H), 1.94-1.52 (comp, 8 H, C3-H & C4-H & C7-H & C7'-H), 1.40-1.24 (comp, 8 H, C8-H & C8'-H & C9-H & C9'-H), 0.97-0.85 (comp, 6 H, C10-H & C10'-H); ^{13}C NMR (75 MHz, CD_3OD/TFA-*d*) δ 175.03 (C5'), 174.97 (C5), 169.2 (C13), 72.9 (d, J = 17.5 Hz, C2), 65.5 (C1), 63.2 (d, J = 6.0 Hz, C11),

54.6 (d, *J* = 4.9 Hz, C12), 35.1 (C6'), 34.9 (C6), 32.4 (C7 & C7'), 25.7 (C8'), 25.6 (C8), 25.5 (d, *J* = 0.5 Hz, C3), 23.4 (C9 & C9'), 23.1 (d, *J* = 139.3, C4), 14.3 (C10 & C10').

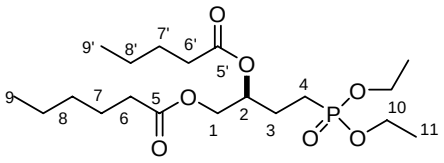


3.51a

Diethyl 3(*S*),4-dibutyroyloxybutyl-1-phosphonate (3.51a). To a mixture of butyric acid (254 mg, 2.90 mmol), diol **3.50** (297 mg, 1.31 mmol) and DMAP (36 mg, 0.29 mmol) in CH_2Cl_2 (5 mL) at 0 °C, was added DCC (619 mg, 3.0 mmol) in CH_2Cl_2 (3.5 mL). The mixture was stirred at rt overnight. The insoluble solid was removed by filtration and washed with CH_2Cl_2. The combined filtrate and wash were concentrated under reduced pressure. The residue was redissolved in ether and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by chromatography eluting with EtOAc/hexanes (from 1:1 to 4:1) to yield 267 mg (56%) of **3.50** as a clear liquid: ^{1}H NMR (250 MHz) δ 4.98-4.89 (m, 1 H), 4.09 (dd, *J* =11.9, 3.8 Hz, 1 H), 4.01-3.83 (comp, 5 H), 2.13 (t, *J* = 7.3 Hz, 2 H), 2.12 (t, *J* = 7.3 Hz, 2 H), 1.80-1.40 (comp, 8 H), 1.16 (t, *J* = 7.1 Hz, 6 H), 0.78 (t, *J* = 7.4 Hz, 3 H), 0.77 (t, *J* = 7.4 Hz, 3 H); ^{13}C NMR (62 MHz) δ 172.9, 172.7, 70.6 (d, *J* = 18.2 Hz), 64.0, 61.4 (d , *J* = 6.5 Hz), 35.9, 35.7, 23.9 (d, *J* = 4.1 Hz), 21.4 (d, *J* = 143.6 Hz), 18.2, 18.1, 16.2, 16.1, 13.4; ^{31}P NMR (121 MHz) δ 31.9; IR (neat) 2967, 1758 cm^{-1}; mass spectrum (CI) m/z 367.1876 [$C_{16}H_{32}NO_7P$ (M+1) requires 367.1886] (base), 297, 279.

NMR assignments. ^{1}H NMR (250 MHz) δ 4.98-4.89 (m, 1 H, C2-H), 4.09 (dd, *J* = 11.9, 3.8 Hz, 1 H, C1-H), 4.01-3.83 (comp, 5 H, C1-H & C9-H), 2.13 (t, *J* = 7.3 Hz, 2 H, C6'-H), 2.12 (t, *J* = 7.3 Hz, 2 H, C6-H), 1.80-1.40 (comp, 8 H, C3-H & C4-H & C7-H

& C7'-H), 1.16 (t, *J* = 7.1 Hz, 6 H, C10-H), 0.78 (t, *J* = 7.4 Hz, 3 H, C8'-H), 0.77 (t, *J* = 7.4 Hz, 3 H, C8-H); ^{13}C NMR (62 MHz) δ 172.9 (C5'), 172.7 (C5), 70.6 (d, *J* = 18.2 Hz, C2), 64.0 (C1), 61.4 (d, *J* = 6.5 Hz, C9), 35.9 (C6'), 35.7 (C6), 23.9 (d, *J* = 4.1 Hz, C3), 21.4 (d, *J* = 143.6 Hz. C4), 18.2 (C7'), 18.1 (C7), 16.2 (d, *J* = 5.8 Hz, C10), 13.4 (C8 & C8').

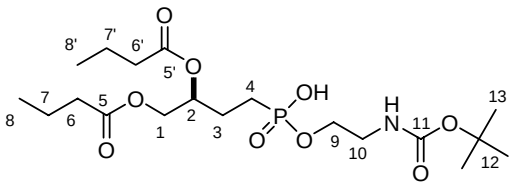


3.51b

Diethyl 3(*S*),4-divaleroxyloxybutyl-1-phosphonate (3.51b). By applying the same method to make **3.51a,** diester **3.51b** was prepared (80%) as a pale yellow liquid: ^{1}H NMR (300 MHz) δ 5.09-5.01 (m, 1 H), 4.20 (dd, *J* = 11.8, 3.8 Hz, 1 H), 4.12-3.98 (comp, 4 H), 3.99 (dd, *J* = 11.8, 5.9 Hz, 1 H), 2.29-2.23 (comp, 4 H), 1.92-1.63 (comp, 4 H), 1.63-1.49 (comp, 4 H), 1.38-1.17 (comp, 10 H), 0.87 (t, *J* = 7.2 Hz, 3 H), 0.86 (t, *J* = 7.2 Hz, 3 H); ^{13}C NMR (75 MHz) δ 173.4, 173.2, 70.9 (d, *J* = 18 Hz), 64.3, 61.8 (d, *J* = 6.6 Hz), 60.4, 34.1, 34.9, 27.1, 27.0, 24.2 (d, *J* = 4.4 Hz), 21.8 (d, *J* = 142.6 Hz), 22.3, 16.6 (d, *J* = 6.0 Hz), 13.8; ^{31}P NMR (121 MHz) δ 31.8.

NMR assignments. ^{1}H NMR (300 MHz) δ 5.09-5.01 (m, 1 H, C2-H), 4.20 (dd, *J* = 11.8, 3.8 Hz, 1 H, C1-H), 4.12-3.98 (comp, 4 H, C10-H), 3.99 (dd, *J* = 11.8, 5.9 Hz, 1 H, C1-H), 2.29-2.23 (comp, 4 H, C6-H & C6'-H), 1.92-1.63 (comp, 4 H, C3-H & C4-H), 1.63-1.49 (comp, 4 H, C7-H & C7'-H), 1.38-1.17 (comp, 10 H, C11-H & C8-H & C8'-H), 0.87 (t, *J* = 7.2 Hz, 3 H, C9'-H), 0.86 (t, *J* = 7.2 Hz, 3 H, C9-H); ^{13}C NMR (75 MHz) δ 173.4 (C5'), 173.2 (C5), 70.9 (d, *J* = 18 Hz, C2), 64.3 (C1), 61.8 (d, *J* = 6.6 Hz, C10), 34.1 (C6'), 34.9 (C6), 27.1 (C7'), 27.0 (C7), 24.2 (d, *J* = 4.4 Hz, C3), 22.3 (C8 & C8'),

21.8 (d, *J* = 142.6 Hz, C4), 16.6 (d, *J* = 6.0 Hz, C11), 13.8 (C9 & C9'); ^{31}P NMR (121 MHz) δ 31.8;



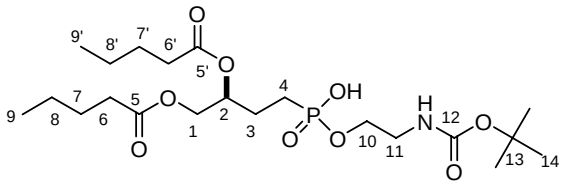
3.52a

***N-tert*-Butoxycarbonyl-3(*S*),4-dibutyroyloxybutyl-1-phosphonylethanolamine (3.52a).** To a solution of **3.51a** (208 mg, 0.57 mmol) in dry CH_2Cl_2 (1 mL), was added bromotrimethylsilane (195 μL, 1.48 mmol) at rt over 30 min. The solution was stirred for 6 h at rt. The solvent was removed under reduced pressure, and THF/H_2O (1 mL, 1:9) was added. The mixture was heated at reflux for 1 h. Solvent was removed under reduced pressure, and the residual oil was dissolved in $CHCl_3$ (5 mL), dried (Na_2SO_4), filtered and evaporated to yield 160 mg of phosphonic acid as pale yellow liquid.

By applying the same method to make **3.52c, 3.52a** was prepared from phosphonic acid (43%) as a yellow glass:^{1}H NMR (300 MHz) δ 6.1-5.7 (br s, 1 H), 5.20-5.00 (br, s, 1 H), 4.30 (d, *J* = 10.8 Hz, 1 H), 4.03-3.97 (m, 1 H), 3.86 (s, 2 H), 3.40-3.10 (br s, 2 H), 2.32-2.22 (m, 4 H), 1.88-1.72 (br s, 2 H), 1.68-1.56 (comp, 6 H), 1.43 (s, 9 H), 0.94 (t, *J* = 7.4 Hz, 3 H), 0.93 (t, *J* = 7.3 Hz, 3 H); ^{13}C NMR (75 MHz) δ 173.6, 156.8, 79.4, 72.1 (d, *J* = 15.8 Hz), 65.0, 63.9, 41.7, 36.5, 36.2, 28.7, 25.2, 22.4 (d, *J*= 136.5 Hz), 18.6, 18.5, 13.9; ^{31}P NMR (121 MHz) δ 25.1 (br); mass spectrum (CI) m/z 454.2216 [$C_{19}H_{37}NO_9P$ (M+1) requires 454.2206] (base), 398, 354, 310, 282.

NMR assignments. ^{1}H NMR (300 MHz) δ 6.1-5.7 (br s, 1 H, NH), 5.20-5.00 (br, s, 1 H, C2-H), 4.30 (d, *J* = 10.8 Hz, 1 H, C1-H), 4.03-3.97 (m, 1 H, C1-H), 3.86 (s, 2 H, C9-H), 3.40-3.10 (br s, 2 H, C10-H), 2.32-2.22 (m, 4 H, C6-H & C6'-H), 1.88-1.72 (br s,

2 H, C3-H), 1.68-1.56 (comp, 6 H, C4-H & C7-H & C7'-H), 1.43 (s, 9 H, C13-H), 0.94 (t, *J* = 7.4 Hz, 3 H, C8'-H), 0.93 (t, *J* = 7.3 Hz, 3 H, C8-H); ^{13}C NMR (75 MHz) δ 173.6 (C5 & C5'), 156.8 (C11), 79.4 (C12), 72.1 (d, *J* = 15.8 Hz, C2), 65.0 (C1), 63.9 (C9), 41.7 (C10), 36.5 (C6'), 36.2 (C6), 28.7 (C13), 25.2 (C3), 22.4 (d, *J*= 136.5 Hz, C4), 18.6 (C7'), 18.5 (C7), 13.9 (C8 & C8').

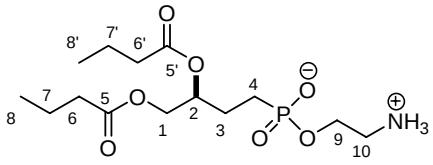


3.52b

***N-tert*-Butoxycarbonyl-3(*S*),4-divaleroxyloxybutyl-1-phosphonylethanolamine (3.52b).** By applying the same method to make **3.52c, 3.52b** was prepared from **3.51b** (19% after two steps) as a yellow glass:^{1}H NMR (250 MHz) δ 6.0-5.8 (br s, 1 H), 5.18-5.00 (m, 1 H), 4.30-4.16 (m, 1 H), 3.95 (dd, *J* = 11.8, 7.4 Hz, 1 H), 3.85-3.7 (br s, 2 H), 3.4-3.1 (br s, 2 H), 2.28-2.20 (comp, 4 H), 1.85-1.65 (br, s, 2 H), 1.65-1.45 (comp, 6 H), 1.38 (s, 9 H), 1.35-1.19 (comp, 4 H), 0.86 (t, *J* = 7.3 Hz, 3 H), 0.85 (t, *J* = 7.2 Hz, 3 H); ^{13}C NMR (62 MHz) δ 173.8, 156.8, 79.4, 72.1 (d, *J* = 15.2 Hz), 65.0, 64.1, 41.7, 34.3, 34.0, 28.7, 27.2, 27.1, 25.2, 22.3 (d, *J* = 140.4 Hz), 22.4, 13.9; ^{31}P NMR (121 MHz) δ 25.8; mass spectrum (CI) m/z 482.2524 [$C_{21}H_{41}NO_9P$ (M+1) requires 482.2519], 442, 351 (base), 324, 280.

NMR assignments. ^{1}H NMR (250 MHz) δ 6.0-5.8 (br s, 1 H, N-H), 5.18-5.00 (m, 1 H, C2-H), 4.30-4.16 (m, 1 H, C1-H), 3.95 (dd, *J* = 11.8, 7.4 Hz, 1 H, C1-H), 3.85-3.7 (br, s, 2 H, C10-H), 3.4-3.1 (br s, 2 H, C11-H), 2.28-2.20 (comp, 4 H, C6-H & C6'-H), 1.85-1.65 (br, s, 2 H, C3-H), 1.65-1.45 (comp, 6 H, C4-H & C7-H & C7'-H), 1.38 (s, 9 H, C14-H), 1.35-1.19 (comp, 4 H), 0.86 (t, *J* = 7.3 Hz, 3 H, C9'-H), 0.85 (t, *J* = 7.2 Hz,

3 H, C9-H); ^{13}C NMR (62 MHz) δ 173.8 (C5 & C5'), 156.8 (C12), 79.4 (C13), 72.1 (d, *J* = 15.2 Hz, C2), 65.0 (C1), 64.1 (C10), 41.7 (C11), 34.3 (C6'), 34.0 (C6), 28.7 (C14), 27.2 (C7'), 27.1 (C7), 25.2 (C3), 22.3 (d, *J* = 140.4 Hz, C4), 22.4 (C8 & C8'), 13.9 (C9 & C9')

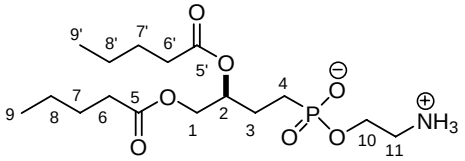


3.53a

3(*S*),4-Dibutyroyloxybutyl-1-phosphonylethanolamine (3.53a). N-Boc protected PE **3.52a** (46 mg, 0.10 mmol) was dissolved in dry CH_2Cl_2 (4.5 mL) containing TFA (0.45 mL) at 0 °C, and the solution was stirred at rt for 45 min. The solvent was removed under reduced pressure, and the residue was purified by chromatography eluting with $CHCl_3$/MeOH (from 3:1 to 2:3) to yield 28 mg (78%) of **3.53a** as a glass:^{1}H NMR (300 MHz) δ 8.80-8.40 (br s, 3 H), 5.20-5.15 (m, 1 H), 4.28 (dd, *J* = 11.8, 2.8 Hz, 1 H), 4.20-4.00 (br s, 2 H), 4.03 (dd, *J* = 11.8, 6.8 Hz, 1 H), 3.20-3.06 (br s, 2 H), 2.33-2.26 (comp, 4 H), 1.90-1.72 (comp, 2 H), 1.72-1.54 (comp, 6 H), 0.96 (t, *J* = 7.4 Hz, 3 H), 0.95 (t, *J* = 7.4 Hz, 3 H); ^{13}C NMR (75 MHz) δ 173.6, 173.5, 71.6 (d, *J* = 16.4 Hz), 64.8, 61.6, 40.7, 36.5, 36.2, 25.2, 22.3 (d, *J* = 137.1 Hz), 18.7, 18.5, 13.9; ^{31}P NMR (121 MHz) δ 27.4; mass spectrum (CI) m/z 354.1682 [$C_{14}H_{29}NO_7P$ (M+1) requires 354.1683] (base), 337, 367, 209.

NMR assignments. ^{1}H NMR (300 MHz) δ 8.80-8.40 (br s, 3 H, NH), 5.20-5.15 (m, 1 H, C2-H), 4.28 (dd, *J* = 11.8, 2.8 Hz, 1 H, C1-H), 4.20-4.00 (br s, 2 H, C9-H), 4.03 (dd, *J* = 11.8, 6.8 Hz, 1 H, C1-H), 3.20-3.06 (br s, 2 H, C10-H), 2.33-2.26 (comp, 4 H, C6-H & C6'-H), 1.90-1.72 (comp, 2 H, C3-H), 1.72-1.54 (comp, 6 H, C4-H & C7-H &

C7'-H), 0.96 (t, *J* = 7.4 Hz, 3 H, C8'-H), 0.95 (t, *J* = 7.4 Hz, 3 H, C8-H); ^{13}C NMR (75 MHz) δ 173.6 (C5'), 173.5 (C5), 71.6 (d, *J* = 16.4 Hz, C2), 64.8 (C1), 61.6 (C9), 40.7 (C10), 36.5 (C6'), 36.2 (C6), 25.2 (C3), 22.3 (d, *J* = 137.1 Hz, C4), 18.7 (C7'), 18.5 (C7), 13.9 (C8 & C8').

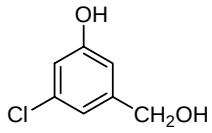


3.53b

3(*S*),4-divaleroxyloxybutyl-1-phosphonylethanolamine (3.53b). By applying the same method to make **3.53a,** PE **3.53b** was prepared from **3.52b** (74%) as glass:^{1}H NMR (300 MHz) δ 8.90-8.50 (br s, 3 H), 5.20-5.12 (m, 1 H), 4.27 (dd, *J* = 11.8, 6.9 Hz, 1 H), 4.14-3.94 (comp, 3 H), 3.18-3.04 (br s, 2 H), 2.32 (t, *J* = 7.5 Hz, 2 H), 2.30 (t, *J* = 7.4 Hz, 2 H), 1.90-1.74 (comp, 2 H), 1.70-1.50 (comp, 6 H), 1.42-1.26 (comp, 4 H), 0.93 (t, *J* = 7.4 Hz), 0.92 (t, *J* =7.2 Hz); ^{13}C NMR (75 MHz) δ 173.8, 173.7, 71.7 (d, *J* = 15.3 Hz), 64.9, 61.3, 40.6, 34.3, 34.0, 27.3, 27.1, 25.4 (d, *J* = 3.8 Hz), 22.4, 22.4 (d, *J* = 136.6 Hz), 14.0, 13.9; ^{31}P NMR (121 MHz) δ 27.3; mass spectrum (CI) m/z 382.2002 [$C_{16}H_{33}NO_7P$ (M+1) requires 382.1994] (base), 364.

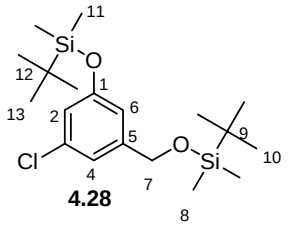
NMR assignments. ^{1}H NMR (300 MHz) δ 8.90-8.50 (br s, 3 H, NH), 5.20-5.12 (m, 1 H, C2-H), 4.27 (dd, *J* = 11.8, 6.9 Hz, 1 H, C1-H), 4.14-3.94 (comp, 3 H, C1-H & C10-H), 3.18-3.04 (br s, 2 H, C11-H), 2.32 (t, *J* = 7.5 Hz, 2 H, C6'-H), 2.30 (t, *J* = 7.4 Hz, 2 H, C6-H), 1.90-1.74 (comp, 2 H, C3-H), 1.70-1.50 (comp, 6 H, C4-H & C7-H & C7'-H), 1.42-1.26 (comp, 4 H, C8-H & C8'-H), 0.93 (t, *J* = 7.4 Hz, C9'-H), 0.92 (t, *J* =7.2 Hz, C9-H); ^{13}C NMR (75 MHz) δ 173.8 (C5'), 173.7 (C5), 71.7 (d, *J* = 15.3 Hz,

C2), 64.9 (C1), 61.3 (C10), 40.6 (C11), 34.3 (C6'), 34.0 (C6), 27.3 (C7'), 27.1 (C7), 25.4 (d, J = 3.8 Hz, C3), 22.4 (d, J = 136.6 Hz, C4), 22.4 (C8), 14.0 (C9'), 13.9 (C9).



4.27

3-Chloro-5-(hydroxymethyl)phenol (4.27). (2-189). Methyl ester **4.26** (180 mg, 0.97 mmol) was suspended in H_2O/dioxane (5 mL, 4:1), and solid $NaBH_4$ (280 mg, 7.4 mmol) was added. The solution was stirred at rt overnight. The mixture was cooled to 0 °C and 2 N HCl (7 mL) was added. The aqueous mixture was extracted with EtOAc (3 x 15 mL). The combined organic phases were dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by chromatography eluting with EtOAc/hexanes (1:1) to yield 125 mg (81%) of **4.27** as a clear liquid: ^{1}H NMR δ (250 MHz, acetone-d_6) δ 9.00-8.50 (br s, 1 H), 6.87-6.83 (m, 1 H), 6.81-6.77 (m, 1 H), 6.75-6.71 (m, 1 H), 4.56 (s, 3 H); ^{13}C NMR δ (62 MHz, acetone-d_6) δ 159.0, 146.5, 134.7, 118.2, 114.6, 112.7, 63.8; mass spectrum (CI) *m/z* 159.0220 [$C_7H_8O_2Cl$ (M+1) requires 159.0213], (base), 141.

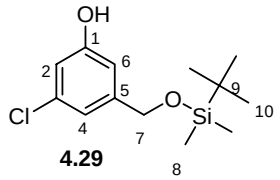


4.28

1-*tert*-Butyldimethylsilanyloxy-3-(*tert*-butyldimethylsilanyloxymethyl)-5-chlorobenzene (4.28). (2-199). A solution of TBSCl (130 mg, 0.84 mmol) in CH_2Cl_2 (2 mL) was added dropwise to a solution of phenol **4.27** (60 mg, 0.38 mmol) and DBU (139

mg, 0.91 mmol) in dry CH_2Cl_2 (3 mL) at 0 °C. The solution was stirred at rt for 90 min, and then poured into a mixture of cold H_2O (10 mL) and CH_2Cl_2 (5 mL). The organic layer was separated and washed with 0.1 N HCl (10 mL), saturated $NaHCO_3$ (10 mL), and dried (Na_2SO_4). The solvent was removed under reduced pressure to yield 129 mg (88%) of **4.28** as a clear liquid: ^{1}H NMR (250 MHz) δ 6.85-6.82 (m, 1 H), 6.71-6.67 (comp, 2 H), 4.64 (s, 2 H), 0.97 (s, 9 H), 0.94 (s, 9 H), 0.19 (s, 6 H), 0.09 (s, 6 H); ^{13}C NMR (75 MHz) δ 156.2, 144.3, 134.1, 118.8 (2 C), 115.7, 64.0, 25.7, 25.5, 18.2, 18.0, -4.6, -5.5; IR (neat) 2956, 2867, 1598, 1577, 1449 cm^{-1}; mass spectrum (CI) *m/z* 1387.1940 [$C_{19}H_{36}O_2SiCl$ (M+1) requires 387.1942], 371, 244 (base).

NMR assignments. ^{1}H NMR (250 MHz) δ 6.85-6.82 (m, 1 H, C4-H), 6.71-6.67 (comp, 2 H, C2-H & C6-H), 4.64 (s, 2 H, C7-H), 0.97 (s, 9 H, C13-H), 0.94 (s, 9 H, C10-H), 0.19 (s, 6 H, C11-H), 0.09 (s, 6 H, C8-H).

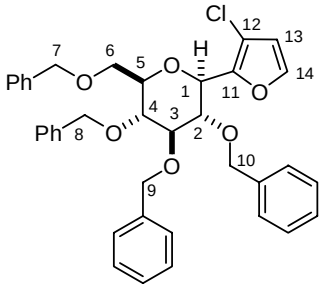


4.29

3-*tert*-Butyldimethylsilanyloxymethyl-5-chlorophenol (4.29). (2-263). A solution of **4.28** (1.11 g, 2.87 mmol) in THF (36 mL) containing 1 N TBAF in THF (2.87 mL, 2.87 mmol) was stirred for 10 min at 0 °C. Saturated NH_4Cl (30 mL) was added and the mixture extracted with Et_2O (3 x 30 mL). The combined organic phases were dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by flash chromatography eluting with EtOAc/hexanes (1:5) to yield 711 mg (91%) of **4.29** as a clear oil: ^{1}H NMR (250 MHz) δ 6.84-6.80 (m, 1 H), 6.70-6.64 (comp, 2 H), 5.07 (s, 1 H), 4.63 (s, 2 H), 0.92 (s, 9 H), 0.09 (s, 6 H); ^{13}C NMR (75 MHz) δ 156.2, 144.8, 134.7,

118.5, 114.3, 111.3, 64.1, 25.9, 18.4, -5.3; IR (neat) 3346 (br), 2951, 1695, 1583, 1461, 1447 cm^{-1}; mass spectrum (CI) *m/z* 273.1085 [$C_{13}H_{22}O_2SiCl$ (M+1) requires 273.1078] (base), 257, 215, 141.

NMR assignments. ^1H NMR (250 MHz) δ 6.84-6.80 (m, 1 H, C4-H), 6.70-6.64 (comp, 2 H, C2-H & C6-H), 5.07 (s, 1 H, OH), 4.63 (s, 2 H, C7-H), 0.92 (s, 9 H, C10-H), 0.09 (s, 6 H, C8-H); ^{13}C NMR (75 MHz) δ 156.2 (C1), 144.8 (C5), 134.7 (C3), 118.5 (C4), 114.3 (C2), 111.3 (C6), 64.1 (C7), 25.9 (C10), 18.4 (C9), -5.3 (C8).



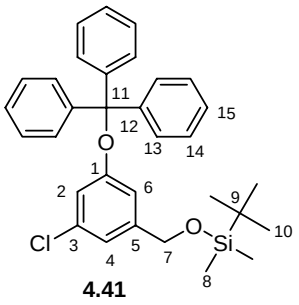
4.33

(2*R*,3R,4S,5R,6R)-3,4,5-tris(benzyloxy)-2-benzyloxymethyl-6-(3-chlorofuran-2-yl)-tetrahydro-2H-pyran (4.33). (2-240). *n*-BuLi in pentane (2.1 M, 1.26 mL, 2.64 mmol) was added to a solution of *i*-Pr_2NH (370 μL, 2.64 mmol) in THF (3 mL) at –10 °C (ice/MeOH bath). The solution was stirred at –10 °C for 25 min and cooled to -78°C, whereupon 3-chlorofuran (246 mg, 2.40 mmol) was added. The solution was stirred at –78 °C for 3.5 h. A solution of glucolactone **4.31** (1.185 g, 2.20 mmol) in THF (6 mL) was added and stirring continued for 2.5 h at –78 °C and then 3 h at rt. The mixture was poured into H_2O (10 mL). The resulting mixture was extracted with ether (2 x 25 mL). The combined organic layers were dried (Na_2SO_4), filtered and concentrated under reduced pressure. The crude material thus obtained was used in the next step without furthur purification: ^1H NMR (300 MHz) δ 7.41-7.08 (comp, 21 H), 6.41 (d, *J* = 1.8 Hz, 1 H), 4.98-4.85 (comp, 3 H), 4.76-4.48 (comp, 5 H), 4.26-3.54 (comp, 8 H); IR

(neat) 3002, 2864, 1726, 1495, 1452 cm^{-1}; mass spectrum (CI) *m/z* 641.2295[$C_{38}H_{38}O_7Cl$ (M+1) requires 625.2306], 623 (base), 533.

The residue was dissolved in CH_2Cl_2 (40 mL) and cooled to –40 °C (dichloroethane/dry ice). Triethylsilane (1.15 mL, 7.20 mmol) and then TMSOTf (434 μL, 2.40 mmol) were added slowly, and the mixture was stirred at –40 °C for 10 min. Saturated $NaHCO_3$ (20 mL) was added and the mixture was extracted with ether (2 x 20 mL). The combined organic layers were dried (Na_2SO_4) and filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by chromatography eluting with EtOAc/hexanes (1:4) to yield 1.037 g (76% after two steps) of **4.33** as a light yellow solid: mp 68.5-72 °C; ^{1}H NMR (300 MHz) δ 7.44-7.03 (comp, 21 H), 6.46 (d, *J* = 1.8 Hz, 6.44 H), 4.98-4.84 (comp, 3 H), 4.68-4.46 (comp, 5 H), 4.10 (d, *J* = 10.2 Hz, 1 H), 4.02-3.92 (m, 1 H), 3.86-3.68 (comp, 4 H), 3.66-3.56 (m, 1 H); ^{13}C NMR (62 MHz) δ 146.2, 142.1, 138.6, 138.1, 138.0, 137.7, 128.4, 128.3, 128.1, 128.06, 127.97, 127.9, 127.7, 127.62, 127.57, 127.5, 115.9, 112.4, 86.6, 80.2, 79.5, 77.9, 75.7, 75.1, 74.7, 73.5, 71.9, 68.9; mass spectrum (CI) *m/z* 625.2339 [$C_{38}H_{38}O_6Cl$ (M+1) requires 625.2357], 533, 433, 391 (base).

NMR assignments. ^{1}H NMR (300 MHz) δ 7.44-7.03 (comp, 21 H, aromatic H & C14-H), 6.46 (d, *J* = 1.8 Hz, 1 H, C13-H), 4.98-4.84 (comp, 3 H, benzylic H), 4.68-4.46 (comp, 5 H, benzylic H), 4.10 (d, *J* = 10.5 Hz, 1 H, C1-H), 4.02-3.92 (m, 1 H, C2-H), 3.86-3.68 (comp, 4 H, C3-H & C4-H & C6-H), 3.66-3.56 (m, 1 H, C5-H).



4.41

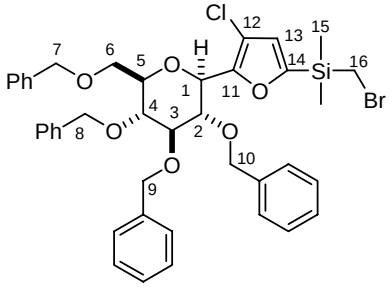
(3-chloro-5-trityloxybenzyloxy)(*tert*-butyl)dimethylsilane (4.41). (2-248, 2-249, 2-251). Trityl bromide (729 mg, 2.26 mmol) in CH_2Cl_2 (5 mL) was added dropwise to a solution of **4.26** (383 mg, 2.05 mmol), Et_3N (430 μL, 3.08 mmol) and DMAP (26 mg, 0.21 mmol) in CH_2Cl_2 (5 mL) at 0 °C. The solution was stirred at 0 °C for 10 min and then at rt for 3 h. The mixture was poured into H_2O (10 mL) and the resulting mixture was extracted with CH_2Cl_2 (2 x 20 mL). The solvent was removed under reduced pressure to yield 872 mg (99%) of **4.40** as a clear liquid.

Methyl ester **4.40** (872 mg, 2.04 mmol) thus obtained in Et_2O (10 mL) was added to a suspension of $LiAlH_4$ (155 mg, 4.08 mmol) in Et_2O (10 mL). The mixture was stirred at 0 °C for 5 min and then at rt for 1 h. The reaction was quenched by addition of EtOAc (1 mL), and the mixture was stirred for 20 min. Saturated NH_4Cl (10 mL) was added. The solid was removed by vacuum filtration and washed with ether. The mixture was separated and the aqueous phase was extracted with Et_2O (3 x 20 mL). The combined organic phases were dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography eluting with hexanes/EtOAc (3:1) to yield 660 mg (81%) of benzylic alcohol as a white foam: ^{1}H NMR (250 MHz) δ 7.50-7.41 (comp, 6 H), 7.31-7.17 (comp, 9 H), 6.79-6.76 (m, 1 H), 6.68-6.64 (m, 1 H), 6.60-6.56 (m, 1 H), 4.31 (s, 2 H), 2.10-1.95 (br s, 1 H); ^{13}C NMR (62 MHz) δ 157.0,

143.5, 142.5, 133.6, 128.7, 127.8, 127.3, 120.1, 119.7, 117.4, 91.0, 64.1; mass spectrum (CI) *m/z* 401.1305 [$C_{26}H_{22}O_2Cl$ (M+1) requires 401.1308], 383 (base).

By applying the same method to make **4.28**, **4.41** was prepared (94%) as a white solid from benzylic alcohol obtained above (634 mg, 1.59 mmol), DBU (320 mg, 2.10 mmol) and TBSCl (288 mg, 1.91 mmol) in CH_2Cl_2 (10 mL): mp 69.5-71.5 °C: ^{1}H NMR (250 MHz) δ 7.45-7.37 (comp, 6 H), 7.30-7.15 (comp, 9 H), 6.73-6.70 (m, 1 H), 6.60-6.56 (m, 1 H), 6.53-6.48 (m, 1 H), 4.40 (s, 2 H), 0.84 (s, 9 H), -0.03 (s, 6 H); ^{13}C NMR (62 MHz) δ 156.9, 143.7, 143.0, 133.4, 128.8, 127.8, 127.3, 119.7, 119.1, 116.7, 90.9, 64.0, 25.9, 18.3, -5.4**;** IR ($CHCl_3$) 1953, 2853, 1578, 1445 cm^{-1}; mass spectrum (CI) *m/z* 513.2013 [$C_{32}H_{334}O_2SiCl$ (M+1) requires 519.2017], 437, 383, 243 (base).

NMR assignments. ^{1}H NMR (250 MHz) δ 7.45-7.37 (comp, 6 H, C14-H), 7.30-7.15 (comp, 9 H., C13-H & C15-H), 6.73-6.70 (m, 1 H, C4-H), 6.60-6.56 (m, 1 H, C2-H), 6.53-6.48 (m, 1 H, C6-H), 4.40 (s, 2 H, C7-H), 0.84 (s, 9 H, C10-H), -0.03 (s, 6 H, C8-H).

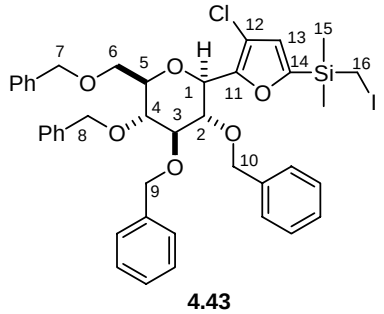


4.42

(5-(2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5-trisbenzyloxy-6-benzyloxymethyl-tetrahydro-2H-pyran-2-yl-4-chlorofuran-2-yl)(bromomethyl)dimethylsilane (4.42). (2-274). A solution of LDA in THF (0.48 M, 5.8 mL, 2.77 mmol) was added to **4.33** (1.019 g, 1.63 mmol) in THF (12 mL) at –78 °C. The mixture was stirred at –78 °C for 2.5 h and then –

38 °C for 4.5 h. The solution was cooled down to –78 °C and bromomethylchlorodimethylsilane (380 μL, 2.77 mmol) was then added dropwise, the mixture was then allowed to warm to rt and stirred overnight. The mixture was poured into $NaHCO_3$ (1:1, 20 mL), and the resulting mixture was extracted with CH_2Cl_2 (3 x 20 mL). The organic layers were combined, dried (Na_2SO_4)., filtered and concentrated under reduced pressure. The residue was purified by chromatography eluting with Et_2O/hexanes (1:7) to yield 721 mg (56%, 71% brsm) of **4.42** as a clear liquid and 198 mg of **4.33**: ^1H NMR (250 MHz) δ 7.34-7.12 (comp, 20 H), 6.71 (s, 1 H), 4.92-4.79 (comp, 3 H), 4.65-4.40 (comp, 5 H), 4.03 (d, *J* = 10.4 Hz, 1 H), 4.04-3.92 (m, 1 H), 3.81-3.68 (comp, 4 H), 3.62-3.50 (m, 1 H), 2.53 (s, 2 H), 0.37 (s, 3 H), 0.36 (s, 3 H); ^{13}C NMR (62 MHz) δ 156.8, 150.9, 138.6, 138.2, 138.1, 137.8, 128.4, 128.3, 128.0, 127.82, 127.78, 127.7, 127.62, 127.56, 123.3, 116.1, 86.6, 80.5, 79.6, 77.9, 75.7, 75.1, 74.8, 73.5, 72.2, 68.8, 14.9, -4.37, -4.42; IR (neat) 3028, 2867, 1584, 1495, 1452 cm^{-1}; mass spectrum (CI) *m/z* 774.1778 [$C_{41}H_{44}O_6SiClBr$ (M) requires 774.1779] 433, 391 (base).

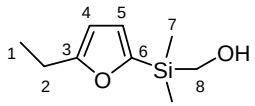
NMR assignments. ^1H NMR (250 MHz) δ 7.34-7.12 (comp, 20 H, aromatic H), 6.71 (s, 1 H, C13-H), 4.92-4.79 (comp, 3 H, benzylic H), 4.65-4.40 (comp, 5 H, benzylic H), 4.03 (d, *J* = 10.4 Hz, 1 H, C1-H), 4.04-3.92 (m, 1 H, C2-H), 3.81-3.68 (comp, 4 H, C3-H & C4-H & C6-H), 3.62-3.50 (m, 1 H, C5-H), 2.53 (s, 2 H, C16-H), 0.37 (s, 3 H, C15-H), 0.36 (s, 3 H, C15-H).



4.43

(5-(2R,3R,4S,5R,6R)-3,4,5-trisbenzyloxy-6-benzyloxymethyl-tetrahydro-2H-pyran-2-yl-4-chlorofuran-2-yl)(iodomethyl)dimethylsilane (4.43). (2-275). A solution of **4.42** (279 mg, 0.36 mmol) and NaI (432 mg, 2.88 mmol) in acetone (12 mL) was heated under reflux overnight. The mixture was poured into H_2O (20 mL), and the resulting mixture was extracted with Et_2O (3 x 20 mL). The organic layers were combined, dried (Na_2SO_4). filtered and concentrated under reduced pressure. The residue was purified by chromatography eluting with Et_2O/hexanes (1:7) to yield 284 mg (96%) of **4.43** as a clear liquid: ^{1}H NMR (300 MHz) δ 7.44-7.04 (comp, 20 H), 6.78 (s, 1 H), 5.02-4.84 (comp, 3 H), 4.78-4.52 (comp, 5 H), 4.13 (d, *J* = 10.5 Hz, 1 H), 4.10-4.04 (m, 1 H), 3.90-3.76 (comp, 4 H), 3.74-3.60 (m, 1 H), 2.16 (s, 2 H), 0.46 (s, 3 H), 0.45 (s, 3 H); ^{13}C NMR (75 MHz) δ 157.3, 151.1, 138.9, 138.4, 138.1, 128.7, 128.6, 128.3, 128.14, 128.06, 127.9, 123.4, 116.3, 86.9, 80.8, 79.9, 78.2, 76.0, 75.4, 75.1, 73.8, 72.5, 69.1, -3.0; IR (neat) 3026, 2863, 1495, 1452 cm^{-1}; mass spectrum (CI) *m/z* 823.1731 [$C_{41}H_{45}O_6SiClI$ (M+1) requires 823.1718] 733, 433, 391 (base).

NMR assignments. ^{1}H NMR (300 MHz) δ 7.44-7.04 (comp, 20 H, aromatic H), 6.78 (s, 1 H, C13-H), 5.02-4.84 (comp, 3 H, benzylic H), 4.78-4.52 (comp, 5 H, benzylic H), 4.13 (d, *J* = 10.5 Hz, 1 H, C1-H), 4.10-4.04 (m, 1 H, C2-H), 3.90-3.76 (comp, 4 H, C3-H & C4-H & C6-H), 3.74-3.60 (m, 1 H, C5-H), 2.16 (s, 2 H, C16-H), 0.46 (s, C15-H), 0.45 (s, C15-H).



4.58

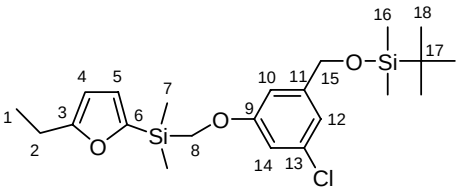
(5-ethylfuran-2-yl)dimethylsilylmethanol (4.58). A 2.1 M solution of BuLi (3.8 mL, 8.0 mmol) was added to 2-ethylfuran (700 mg, 7.29 mmol) in THF (17 mL) at –78 °C. The mixture was stirred at –78 °C for 5 min and then at 0 °C for 1 h. The solution was cooled down to –78 °C and freshly distilled bromomethylchlorodimethylsilane (1 mL, 7.3 mmol) was added dropwise, the mixture was then allowed to warm to rt and stirred for 3.5 h. The mixture was poured into $NaHCO_3$ (1:1, 20 mL), and the resulting mixture was extracted with ether (3 x 25 mL). The organic layers were combined, dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue was purified by chromatography eluting with hexanes to yield 1.335 g (74%) of bromide **4.57** as a clear liquid: ^{1}H NMR (250 MHz) δ 6.62 (d, *J* = 3.1 Hz, 1 H), 5.98 (d, *J* = 3.1 Hz, 1 H), 2.66 (q, *J* = 7.5 Hz, 2 H), 2.60 (s, 2 H), 1.22 (t, *J* = 7.5 Hz, 3 H), 0.38 (s, 6 H); ^{13}C NMR (62 MHz) δ 163.1, 154.3, 122.3, 104.3, 21.5, 16.2, 12.1, -4.3.

A solution of bromide **4.57** (500 mg, 2.02 mmol), KOAc (1.07 g, 11.0 mmol) and NaI (303 mg, 2.02 mmol) in DMF (10 mL) was heated at 70 °C for 4.5 h. The mixture was poured into H_2O (30 mL), and the resulting mixture was extracted with ether (2 x 20 mL). The combined organic layers was washed with brine (30 mL) and dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue was purified by chromatography eluting with Et_2O/hexanes (1:6) to yield 407 mg (89%) of the acetate as a clear liquid: ^{1}H NMR (250 MHz) δ 6.59 (d, *J* = 3.1 Hz, 1 H), 5.96 (d, *J* = 3.1 Hz, 1 H), 3.91 (d, 3 H), 2.65 (q, *J* = 7.5 Hz, 2 H), 2.02 (s, 3 H), 1.21 (t, *J* = 7.5 Hz, 3 H), 0.30 (s, 6 H); ^{13}C NMR (62 MHz) δ 171.7, 163.0, 154.2, 122.1, 104.2, 56.3, 21.5, 20.7, 12.0, -4.7; IR ($CHCl_3$) 2971, 1742 cm^{-1}

A solution of acetate (396 mg, 1.75 mmol) in ether (5 mL) was added to a slurry of LAH (210 mg, 5.27 mmol) in ether (20 mL) at 0 ºC. The mixture was stirred at 0 ºC for 10 min. EtOAc (2 mL) was added, stirring continued for another 5 min at 0 ºC, then a

solution of saturated NH_4Cl added and stirring continued for another 5 min at rt. The mixture was filtered and the solids washed with ether and NH_4Cl, The filtrated and the washes were separated and the aqueous layer was extracted with ether (3 x 25 mL). The organic layers were combined, dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue was purified by chromatography eluting with Et_2O/hexanes (2:3) to yield 301 mg (95%) of **4.58** as a clear liquid: ^{1}H NMR (300 MHz) δ 6.62 (d, *J* = 3.1 Hz, 1 H), 5.98 (d, *J* = 3.1 Hz, 1 H), 3.53 (s, 2 H), 2.66 (q, *J* = 7.6 Hz, 2 H), 1.22 (t, *J* = 7.6 Hz, 3 H), 1.30-1.10 (br s, 1 H), 0.30 (s, 6 H); ^{13}C NMR (62 MHz) δ 163.3, 155.2, 122.5, 104.5, 55.1, 21.8, 12.3, -4.9; mass spectrum (CI) *m/z* 185.0992 [$C_9H_{17}O_2Si$ (M+1) requires 185.0998], 151 (base).

NMR assignments. ^{1}H NMR (300 MHz) δ 6.62 (d, *J* = 3.1 Hz, 1 H, C5-H), 5.98 (d, *J* = 3.1 Hz, 1 H, C4-H), 3.53 (s, 2 H, C8-H), 2.66 (q, *J* = 7.6 Hz, 2 H, C2-H), 1.22 (t, *J* = 7.6 Hz, 3 H, C1-H), 1.30-1.10 (br s, 1 H, O-H), 0.30 (s, 6 H, C7-H);^{13}C NMR (62 MHz) δ 163.3 (C3), 155.2 (C6), 122.5 (C5), 104.5 (C4), 55.1 (C8), 21.8 (C2), 12.3 (C1), -4.9 (C7).

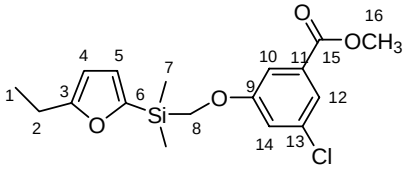


4.59

2-(3-(*tert*-butyldimethylsilanyloxymethyl)-5-chlorophenoxymethyldimethyl-silanyl)-5-ethylfuran (4.59). ADDP (100.3 mg, 0.398 mmol) was added to a solution of alcohol **4.58** (50 mg, 0.27 mmol), phenol **4.29** (75.5 mg, 0.277 mmol) and tributylphosphine (100 μL, 0.0405 mmol) in benzene (1 mL) at 0 °C. The mixture was stirred overnight and filtered through a short column, eluting with Et_2O/hexane (1:10),

the fraction was collected, evaporated and repurified by chromatography eluting with Et_2O/hexanes (1:100) to yield 87.2 mg (73%) of **4.59** as a clear liquid: ^{1}H NMR (300 MHz) δ 7.24 (m, 1 H), 6.85 (m, 1 H), 6.82 (m, 1 H), 6.64 (d, *J* = 2.8 Hz, 1 H), 5.98 (d, *J* = 3.3 Hz, 1 H), 4.64 (s, 2 H), 3.73 (s, 2 H), 2.67 (q, *J* = 7.4 Hz, 2 H), 1.22 (t, *J* = 7.4 Hz, 3 H), 0.93 (s, 9 H), 0.37 (s, 6 H), 0.08 (s, 6 H); ^{13}C NMR (62 MHz) δ 163.0, 162.1, 154.3, 144.2, 134.5, 122.3, 118.0, 113.0, 110.3, 104.3, 64.4, 60.2, 25.9, 21.5, 18.4, 12.1, -4.8, -5.3; mass spectrum (CI) *m/z* 438.1822 [$C_{22}H_{35}O_3Si_2Cl$ (M) requires 438.1813] 343, 307 (base), 211.

NMR assignments. ^{1}H NMR (300 MHz) δ 7.24 (m, 1 H), 6.85 (m, 1 H), 6.82 (m, 1 H), 6.64 (d, *J* = 2.8 Hz, 1 H, C5-H), 5.98 (d, *J* = 3.3 Hz, 1 H, C4-H), 4.64 (s, 2 H, C15-H), 3.73 (s, 2 H, C8-H), 2.67 (q, *J* = 7.4 Hz, 2 H, C2-H), 1.22 (t, *J* = 7.4 Hz, 3 H, C1-H), 0.93 (s, 9 H, C18-H), 0.37 (s, 6 H, C7-H), 0.08 (s, 6 H, C16-H); ^{13}C NMR (62 MHz) δ 163.0 (C3), 162.1, 154.3 (C6), 144.2, 134.5, 122.3 (C5), 118.0, 113.0, 110.3, 104.3 (C4), 64.4 (C15), 60.2 (C8), 25.9 (C18), 21.5 (C2), 18.4 (C17), 12.1 (C1), -4.8 (C7), -5.3 (C16).

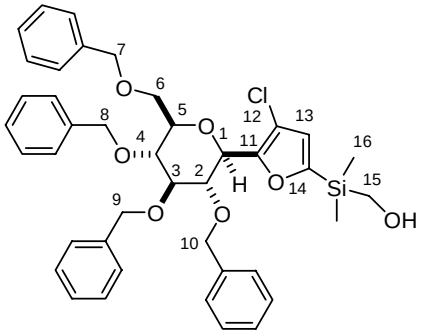


4.60

methyl 3-(5-ethylfuran-2-yl)dimethylsilylmethoxy-5-chlorobenzoate (4.60). (3-56). DIAD (22.2 mg, 1.1 mmol) was added to a solution of alcohol **4.58** (22 mg, 1.2 mmol), phenol **4.26** (19 mg, 1.0 mmol) and triphenylphosphine (31.4 mg, 1.2 mmol) in toluene (0.5 mL). The mixture was stirred at rt for 2 h. the mixture was poured into H_2O (5 mL) and extracted with ether (3 x 5 mL). The combined organic layers were dried

(Na_2SO_4), filtered, and concentrated under reduced pressure. The residue was purified by chromatography eluting first with Et_2O/hexanes (1:100) then with Et_2O/hexanes (1:20) to yield 26 mg (72%) of **4.60** as a clear liquid: ^{1}H NMR (250 MHz) δ 7.55 (m, 1 H), 7.49 (m, 1 H), 7.12 (app t. *J* = 2.0 Hz, 1 H), 6.64 (d, *J* = 3.1 Hz, 1 H), 5.98 (d, *J* = 3.1 Hz, 1 H), 3.89 (s, 3 H), 3.78 (s, 2 H), 2.66 (q, *J* = 7.4 Hz, 2 H), 1.22 (t, *J* = 7.4 Hz, 3 H), 0.38 (s, 6 H); ^{13}C NMR (62 MHz) δ 165.9, 163.2, 162.0, 154.0, 134.9, 132.3, 122.4, 121.7, 119.4, 113.5, 104.3, 60.7, 52.4, 21.5, 12.1, -4.9; mass spectrum (CI) *m/z* 352.0900 [$C_{17}H_{21}O_4SiCl$ (M) requires 352.0898], 257 (base), 241.

NMR assignments. ^{1}H NMR (250 MHz) δ 7.55 (m, 1 H), 7.49 (m, 1 H), 7.12 (app t. *J* = 2.0 Hz, 1 H), 6.64 (d, *J* = 3.1 Hz, 1 H, C5-H), 5.98 (d, *J* = 3.1 Hz, 1 H, C4-H), 3.89 (s, 3 H, C16-H), 3.78 (s, 2 H, C8-H), 2.66 (q, *J* = 7.4 Hz, 2 H, C2-H), 1.22 (t, *J* = 7.4 Hz, 3 H, C1-H), 0.38 (s, 6 H, C7-H); ^{13}C NMR (62 MHz) δ 165.9 (C16), 163.2 (C3), 162.0, 154.0 (C6), 134.9, 132.3, 122.4 (C5), 121.7, 119.4, 113.5, 104.3 (C4), 60.7 (C8), 52.4 (C16), 21.5 (C2), 12.1 (C1), -4.9 (C7).



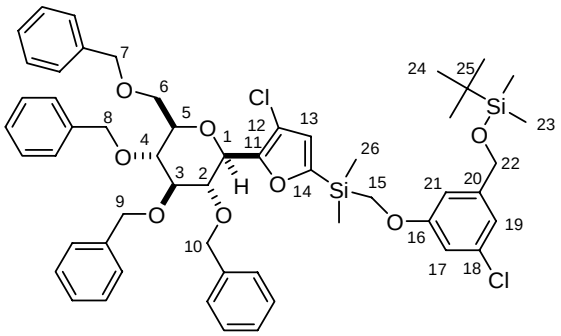
4.62

(5-(2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5-trisbenzyloxy-6-benzyloxymethyl-tetrahydro-2H-pyran-2-yl-4-chlorofuran-2-yl)dimethylsilylmethanol (4.62). (3-63, 3-68) A solution of **4.42** (170 mg, 0.22 mmol), NaOAc (90 mg, 1.1 mmol) and NaI (66 mg, 0.44 mmol) in

DMF (6 mL) was heated at 70 °C for 4 h. The mixture was poured into H_2O (10 mL), and the resulting mixture was extracted with ether (3 x 10 mL). The organic layers were combined, dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue was purified by chromatography eluting with Et_2O/hexanes (1:3) to yield 128 mg (78%) of **4.61** as a clear liquid: mass spectrum (CI) *m/z* 755.2746 [$C_{43}H_{48}O_8SiCl$ (M+1) requires 755.2807] (base), 739, 647.

A solution of acetate **4.61** (276 mg, 0.366 mmol) prepared as mentioned above in ether (5 mL) was added to a slurry of LAH (42 mg, 1.10 mmol) in ether at 0 ºC. The mixture was stirred at 0 ºC for 25 min. EtOAc (1.5 mL) was added, stirring continued for another 5 min at 0 ºC, then a solution of saturated NH_4Cl added and stirring continued for another 5 min at rt. The mixture was filtered and the solids washed with ether and NH_4Cl, The filtrated and the washes were separated and the aqueous layer was extracted with ether (3 x 25 mL). The organic layers were combined, dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue was purified by chromatography eluting with Et_2O/hexanes (2:3) to yield 230 mg (88%) of **4.62** as a clear liquid: ^{1}H NMR (500 MHz, benzene-d_6) δ ^{1}H NMR (500 MHz) δ 7.42-7.00 (comp, 20 H), 6.49 (s, 1 H), 4.97-4.87 (comp, 3 H), 4.70 (d, *J* = 10 Hz, 1 H), 4.69-4.64 (comp, 2 H), 4.46 (d, *J* = 12.0, 1 H), 4.34-4.29 (comp, 2 H), 4.25 (app t, *J* = 9.6, 9.2 Hz, 1 H), 3.92 (app t, *J* = 9.6, 9.2 Hz, 1 H), 3.25 (t, *J* = 9.0 Hz, 1 H), 3.69 (dd, *J* = 11.2, 3.8 Hz, 1 H), 3.58 (dd, *J* = 11.2, 1.6 Hz, 1 H), 3.44 (ddd, *J* = 9.6, 3.8, 1.6 Hz, 1 H), 3.19 (s, 2 H), 0.13 (s, 3 H), 0.12 (s, 3 H); ^{13}C NMR (75 MHz, benzene-d_6) δ 158.3, 151.6, 139.5, 139.2, 138.9, 138.8, 128.4 (3), 127.8 (2), 127.7, 127.5. 123.1, 116.1, 86.9, 80.9, 80.1, 78.2, 75.5, 74.9, 74.7, 73.5, 72.7, 69.1, 53.6, -5.6 (2); mass spectrum (CI) *m/z* 711.2546 [$C_{41}H_{44}O_7SiCl$ (M-1) requires 711.2544], 605 (base), 265.

NMR assignments. ^{1}H NMR (500 MHz, benzene-d_6) δ 7.42-7.00 (comp, 20 H, aromatic H), 6.49 (s, 1 H, C12-H), 4.97-4.87 (comp, 3 H, benzylic H), 4.70 (d, *J* = 10 Hz, 1 H, C1-H), 4.69-4.64 (comp, 2 H, benzylic H), 4.46 (d, *J* = 12.0 Hz, 1 H, benzylic H), 4.34-4.29 (comp, 2 H, benzylic H), 4.25 (app t, *J* = 9.6, 9.2 Hz, 1 H, C2-H), 3.92 (app t, *J* = 9.6, 9.2 Hz, 1 H, C4-H), 3.25 (t, *J* = 9.0 Hz, 1 H, C3-H), 3.69 (dd, *J* = 11.2, 3.8 Hz, 1 H, C6-H), 3.58 (dd, *J* = 11.2, 1.6 Hz, 1 H, C6-H), 3.44 (ddd, *J* = 9.6, 3.8, 1.6 Hz, 1 H, C5-H), 3.19 (s, 2 H, C15-H), 0.13 (s, 3 H, C16-H), 0.12 (s, 3 H, C16-H),; ^{13}C NMR (75 MHz, benzene-d_6) δ 158.3 (C11), 151.6 (C14), 139.5, 139.2, 138.9, 138.8, 128.4 (3), 127.8 (2), 127.7, 127.5 (aromatic C), 123.1 (C12), 116.1 (C13), 86.9 (C3), 80.9 (C2), 80.1 (C5), 78.2 (C4), 75.5, 74.9, 74.7, 73.5 (benzylic C), 72.7 (C1), 69.1 (C6), 53.6 (C15), -5.6 (2, C16)

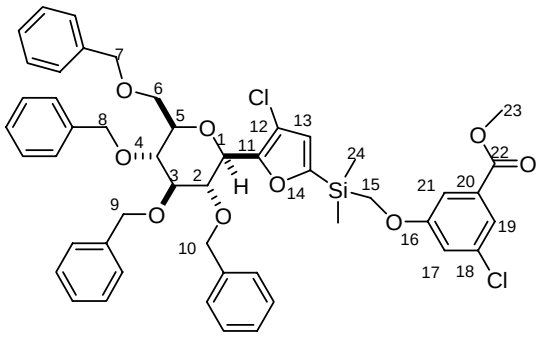


4.63

3,4,5-Tris(benzyloxy)-2-benzyloxymethyl-6-(5-[(3-*tert*-butyldimethylsilanyloxymethyl-5-chlorophenoxymethyl)dimethylsilanyl]-3-chlorofuran-2-yl)-tetrahydropyran (4.63). **(3-71a).** DIAD (9.7 μL, 0.049 mmol) was added to a solution of alcohol **4.62** (38 mg, 0.053 mmol), phenol **4.29** (19 mg, 1.0 mmol) and triphenylphosphine (14 mg, 0.054 mmol) in benzene (0.2 mL). The mixture was stirred at rt for 4 h. and then filtered through a short column, eluting with Et_2O/hexane

(1:3). The fraction was collected, evaporated and repurified by chromatography (Et_3N deactivated silica) eluting with Et_2O/hexanes (1:12) to yield 14.4 mg (31%) of **4.63** as clear liquid: ^{1}H NMR (300 MHz, benzene-d_6) δ 7.40-7.00 (comp, 21 H), 6.78 (comp, 2 H), 6.48 (s, 1 H), 4.98-4.82 (comp, 3 H), 4.70 (d, *J* = 9.9 Hz, 1 H), 4.66-4.60 (comp, 2 H), 4.43 (d, *J* = 12.6 Hz, 1 H), 4.41 (s, 2 H), 4.30-4.18 (comp, 3 H), 3.93 (app t, *J* = 9.3 Hz, 1 H), 3.97-3.38 (comp, 4 H), 3.28 (d, *J* = 12.7 Hz, 1 H), 3.20 (d, *J* = 12.7 Hz, 1 H), 0.93 (s, 9 H), 0.14 (s, 3 H), 0.13 (s, 3 H), 0.00 (s, 6 H); ^{13}C NMR (75 MHz) δ 161.9, 157.2, 151.1, 144.6, 138.9, 138.4, 138.3, 138.0, 134.9, 128.7, 128.6, 128.3, 128.1, 128.0, 127.9 (2), 123.7, 118.6, 116.5, 113.1, 110.4, 86.9, 80.7, 79.8, 78.2, 76.0, 75.4, 75.1, 73.7, 72.5, 69.1, 64.6, 59.6, 26.2, 18.7, -4.8; mass spectrum (CI) *m/z* 966.3498 [$C_{54}H_{64}O_8SiCl_2$ (M+1) requires 966.3517], 827, 279 (base).

NMR assignments. ^{1}H NMR (300 MHz, benzene-d_6) δ 7.40-7.00 (comp, 21 H, aromatic-H), 6.78 (comp, 2 H, aromatic-H), 6.48 (s, 1 H, C12-H), 4.98-4.82 (comp, 3 H, benzylic H), 4.70 (d, *J* = 9.9 Hz, 1 H, C1-H), 4.66-4.60 (comp, 2 H, benzylic H), 4.43 (d, *J* = ~12.6 Hz, 1 H, benzylic H), 4.41 (s, 2 H, C22-H), 4.30-4.18 (comp, 3 H, benzylic H & C1-H), 3.93 (app t, *J* = 9.3 Hz, 1 H, C4-H), 3.97-3.38 (comp, 4 H, C3-H & C6-H & C5-H), 3.28 (d, *J* = 12.7 Hz, 1 H, C15-H), 3.20 (d, *J* = 12.7 Hz, 1 H, C15-H), 0.93 (s, 9 H, C24-H), 0.14 (s, 3 H, C26-H), 0.13 (s, 3 H, C26-H), 0.00 (s, 6 H, C23-H); ^{13}C NMR (75 MHz) δ 161.9, 157.2 (C11), 151.1 (C14), 144.6, 138.9, 138.4, 138.3, 138.0, 134.9, 128.7, 128.6, 128.3, 128.1, 128.0, 127.9 (2), 123.7 (CC12), 118.6, 116.5 (C13), 113.1, 110.4, 86.9 (C3), 80.7(C2), 79.8 (C5), 78.2 (C4), 76.0, 75.4, 75.1, 73.7 (benzylic C), 72.5 (C1), 69.1 (C6), 64.6 (C22), 59.6 (C15), 26.2 (C24), 18.7 (C25), -4.8 (C23).

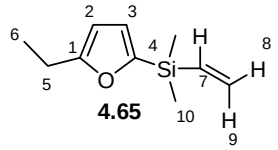


4.64

3-Chloro-5-({[4-chloro-5-(3,4,5-tris(benzyloxy)-6-benzyloxymethyltetrahydropyran-2-yl)-furan-2-yl]-dimethylsilanyl}-methoxy)-benzoic acid methyl ester (4.64). (3-99). By applying similar method to make **4.59,** ether **4.64** was prepared from alcohol **4.62** (41 mg, 0.058 mmol), phenol **4.26** (16.1 mg, 0.086 mmol) and tributylphosphine (21.5 μL, 0.086 mmol) in CH_2Cl_2 (0.25 mL) as a clear liquid (29%, 14.5 mg): ^{1}H NMR (300 MHz) δ 7.59 (m, 1 H), 7.45 (m, 1 H), 7.38-7.27 (comp, 18 H), 7.05 (m, 1 H), 6.97-6.92 (comp, 2 H), 6.78 (s, 1 H), 4.96-4.84 (comp, 3 H), 4.69-4.56 (comp, 3 H), 4.52 (d, *J* = 10.2 Hz, 1 H), 4.51 (d, *J* = 10.5 Hz, 1 H), 4.06 (d, *J* = 10.8 Hz, 1 H), 4.10-4.00 (m, 1 H), 3.90 (s, 3 H), 3.83-3.70 (comp, 6 H), 3.68-3.58 (m, 1 H), 0.41 (s, 6 H); ^{13}C NMR (75 MHz) δ 166.1, 161.9, 156.9, 151.2, 138.8, 138.4, 138.3, 137.9, 135.2, 132.6, 128.7, 128.6, 128.5, 128.3, 128.1, 128.0 (2), 127.9, 127.8, 123.8, 122.2, 119.5, 116.5, 113.6, 86.9, 80.7, 79.8, 78.2, 75.9, 75.4, 75.0, 73.7, 72.4, 69.1, 60.0, 52.7, -4.9, -5.0; mass spectrum (CI) *m/z* 880.2616 [$C_{49}H_{50}O_9SiCl$ (M) requires 880.2601] 559, 433 (base).

NMR assignments. ^{13}C NMR (75 MHz) δ 166.1, 161.9, 156.9, 151.2 (C14), 138.8, 138.4, 138.3, 137.9, 135.2, 132.6, 128.7, 128.6, 128.5, 128.3, 128.1, 128.0 (2), 127.9, 127.8, 123.8 (C12), 122.2, 119.5, 116.5 (C13), 113.6, 86.9 (C3), 80.7 (C2), 79.8

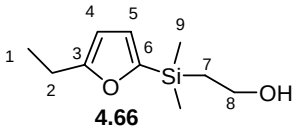
(C5), 78.2 (C4), 75.9, 75.4, 75.0, 73.7 (benzylic-C), 72.4 (C1-H), 69.1 (C6), 60.0 (C23), 52.7 (C23), -4.9 (C24), -5.0 (C24).



4.65

(5-Ethylfuran-2-yl)-dimethylvinylsilane (4.65). (3-151) By applying similar method to make **4.57,** vinylsilane **4.65** was prepared from ethylfuran (1.27 g, 13.2 mmol), BuLi (2.5 M, 5.8 mL, 14.6 mmol) and chlorodimethylvinylsilane (1.82 mL, 13.2 mmol) in THF (25 mL) as a clear liquid (99%, 2.35 g): ^{1}H NMR (250 MHz) δ 6.55 (d, *J* = 3.0 Hz, 1 H), 6.24 (dd, *J* = 20.0, 14.6 Hz, 1 H), 6.02 (dd, *J* = 14.6, 4.0 Hz, 1 H), 5.97 (d, *J* = 3.0 Hz, 1 H), 5.77 (dd, *J* = 20.0, 4.0 Hz, 1 H), 2.68 (q, *J* = 7.6 Hz, 2 H), 1.23 (t, *J* = 7.6 Hz, 3 H), 0.32 (s, 6 H). ^{13}C NMR (62 MHz) δ 162.7, 156.2, 136.9, 133.0, 121.4, 104.0, 21.5, 12.1, -3.3; IR (neat) cm^{-1}; IR (neat) 2966, 1588, 1495, 1402, 1248 cm^{-1}; mass spectrum (CI) *m/z* 181.1045 [$C_{10}H_{17}OSi$ (M+1) requires 181.1049] (base), 165.

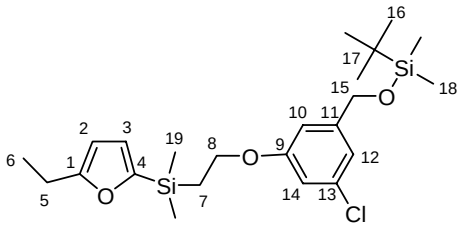
NMR assignments. ^{1}H NMR (250 MHz) δ 6.55 (d, *J* = 3.0 Hz, 1 H, C2-H), 6.24 (dd, *J* = 20.0, 14.6 Hz, 1 H, C7-H), 6.02 (dd, *J* = 14.6, 4.0 Hz, 1 H, C8-H), 5.97 (d, *J* = 3.0 Hz, 1 H, C3-H), 5.77 (dd, *J* = 20.0, 4.0 Hz, 1 H, C9-H), 2.68 (q, *J* = 7.6 Hz, 2 H, C5-H), 1.23 (t, *J* = 7.6 Hz, 3 H, C6-H), 0.32 (s, 6 H, C10-H). ^{13}C NMR (62 MHz) δ 162.7 (C1), 156.2 (C4), 136.9 (C7), 133.0 (C11), 121.4 (C3), 104.0 (C2), 21.5 (C5), 12.1 (C6), -3.3 (C10).



4.66

2-(5-Ethylfuran-2-yl)-dimethylsilanylethanol (4.66). (3-112) A solution of vinylsilane **4.65** (190 mg, 1.05 mmol) and 0.5 M 9-BBN in THF (2.3 mL, 1.15 mmol) was stirred overnight. A 30% NaOH solution (0.6 mL) was added at rt, then 30% H_2O_2 (0.6 mL) was added immediately,. The mixture was stirred at rt for 1 h and then poured in to $NaHCO_3$ (10 mL). The mixture was extracted with ether (3 x 10 mL). The organic layers were combined, dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue was purified by chromatography eluting with Et_2O/hexanes (2:3) to yield 167 mg (80%) of **4.66** as a clear liquid: ^{1}H NMR (300 MHz) δ 6.53 (d, *J* = 3 Hz, 1 H), 5.95 (d, *J* = 3 Hz, 1 H), 3.74 (t, *J* = 8.1 Hz, 2 H), 2.65 (q, *J* = 7.6 Hz, 2 H), 1.21 (t, *J* = 7.6 Hz, 3 H), 1.15 (t, *J* = 8.1 Hz, 2 H), 0.25 (s, 6 H); ^{13}C NMR (75 MHz) δ 162.8, 156.8, 121.5, 104.4, 59.9, 21.8, 21.0, 12.4, -2.8; IR ($CHCl_3$) 3346, 2969, 1588 cm^{-1}; mass spectrum (CI) *m/z* 198.1072 [$C_{10}H_{18}O_2Si$ (M) requires 198.1076], 171, 149.

NMR assignments. ^{1}H NMR (300 MHz) δ 6.53 (d, *J* = 3 Hz, 1 H, C5-H), 5.95 (d, *J* = 3 Hz, 1 H, C4-H), 3.74 (t, *J* = 8.1 Hz, 2 H, C8-H), 2.65 (q, *J* = 7.6 Hz, 2 H, C2-H), 1.21 (t, *J* = 7.6 Hz, 3 H, C1-H), 1.15 (t, *J* = 8.1 Hz, 2 H, C7-H), 0.25 (s, 6 H, C9-H); ^{13}C NMR (75 MHz) δ 162.8 (C3), 156.8 (C6), 121.5 (C5), 104.4 (C4), 59.9 (C8), 21.8 (C2), 21.0 (C7), 12.4 (C1), -2.8 (C9).

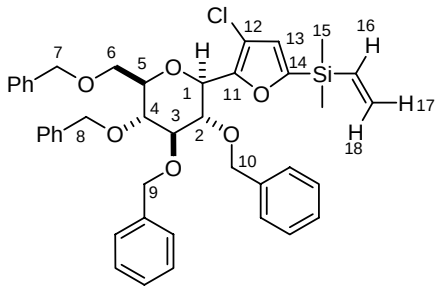


4.67

2-({2-[3-(*tert*-Butyldimethylsilanyloxymethyl)-5-chlorophenoxy]ethyl}dimethylsilanyl)-5-ethylfuran (4.67). (3-124) By applying

similar method to make **4.60**, ether **4.67** was prepared from alcohol **4.66** (71 mg, 0.36 mmol), phenol **4.29** (113 mg, 0.42 mmol) and triphenylphosphine (109 mg, 0.42 mmol) in CH_2Cl_2 (1.5 mL) as a clear liquid (49%, 80 mg): ^{1}H NMR (300 MHz) δ 6.84 (m, 1 H), 6.71 (m, 2 H), 6.56 (d, *J* = 3.0 Hz, 1 H), 5.96 (d, *J* = 3 Hz, 1 H), 4.64 (s, 2 H, C7-H), 4.06 (t, *J* = 8.0 Hz, 2 H), 2.66 (q, *J* = 7.5 Hz, 2 H), 1.33 (t, *J* = 8.0 Hz, 2 H), 1.22 (t, *J* = 7.5 Hz, 3 H), 0.92 (s, 9 H), 0.30 (s, 6 H), 0.08 (s, 6 H); ^{13}C NMR (75 MHz) δ 162.9, 159.8, 156.4, 144.7, 134.8, 121.6, 118.3, 113.5, 110.8, 104.4, 65.7, 64.5, 26.2, 21.8, 18.6, 16.8, 12.4, -2.7, -5.0; IR (benzene) 29254, 2856, 1577, 1452 cm^{-1}; mass spectrum (CI) *m/z* 452.1963 [$C_{23}H_{37}O_3Si_2Cl$ (M) requires 452.1970] (base), 321, 279.

NMR assignments. ^{1}H NMR (300 MHz) δ 6.84 (m, 1 H), 6.71 (m, 2 H), 6.56 (d, *J* = 3.0 Hz, 1 H, C3-H), 5.96 (d, *J* = 3 Hz, 1 H, C2-H), 4.64 (s, 2 H, C15-H), 4.06 (t, *J* = 8.0 Hz, 2 H, C8-H), 2.66 (q, *J* = 7.5 Hz, 2 H, C5-H), 1.33 (t, *J* = 8.0 Hz, 2 H, C7-H), 1.22 (t, *J* = 7.5 Hz, 3 H, C6-H), 0.92 (s, 9 H, C16-H), 0.30 (s, 6 H, C19-H), 0.08 (s, 6 H, C18-H); ^{13}C NMR (75 MHz) δ 162.9 (C1), 159.8, 156.4 (C4), 144.7, 134.8, 121.6 (C3), 118.3, 113.5, 110.8, 104.4 (C2), 65.7 (C8), 64.5 (C15), 26.2 (C16), 21.8 (C6) 18.6 (C17), 16.8 (C7), 12.4 (C6), -2.7 (C19), -5.0 (C18)

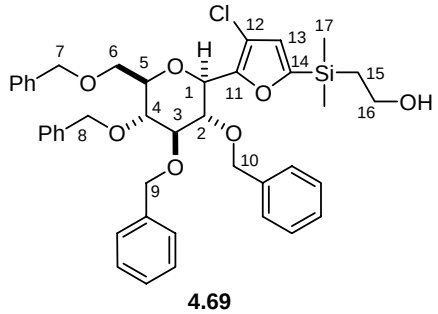


4.68

[4-Chloro-5-(3,4,5-trisbenzyloxy-6-benzyloxymethyltetrahydropyran-2-yl)-furan-2-yl]-dimethylvinylsilane (4.68). (2-265). A 0.42 M solution of LDA in THF (1.38 mL, 0.576 mmol) was added to a solution of **4.33** (180 mg, 0.288 mmol) in THF

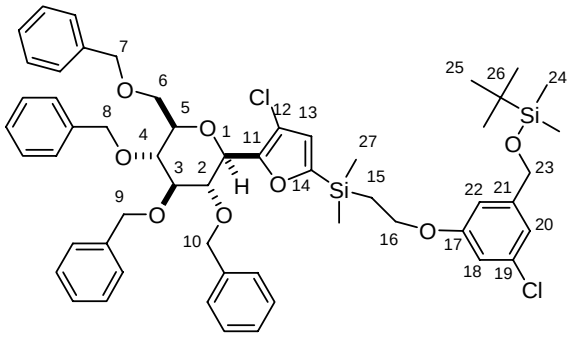
(2.5 mL) at –78 °C. The solution was stirred at –78 °C for 3 h and then at –40 °C for 3 h. The mixture was diluted with $NaHCO_3$ (15 mL, 1:1), and the resulting mixture was extracted with CH_2Cl_2 (3 x 10 mL). The combined organic layers was dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography eluting with Et_2O/hexane (1:7) to yield 92 mg (45%, 61% brsm) of **4.68** as a clear oil and 48 mg of **4.33**: ^{1}H NMR (300 MHz) δ 7.40-6.98 (comp, 20 H), 6.67 (s, 1 H), 6.21 (dd, *J* = 19.6, 14.6 Hz, 1 H), 6.06 (dd, *J* = 14.6, 4.3 Hz, 1 H), 5.81 (d, *J* = 19.6, 4.3 Hz, 1 H), 5.00-4.85 (comp, 3 H), 4.72-4.48 (comp, 5 H), 4.10 (d, *J* = 10.5 Hz, 1 H), 4.12-4.00 (m, 1 H), 3.88-3.70 (comp, 4 H), 3.70-3.58 (m, 1 H) 0.35 (s, 6 H); ^{13}C NMR (75 MHz) δ 158.8, 150.4, 138.6, 138.2, 138.1, 137.8, 135.4, 134.0, 128.3, 128.2, 127.91, 127.85, 127.72, 127.67, 127.54, 127.46, 122.5, 115.9, 86.5, 80.4, 79.5, 77.9, 75.6, 75.0, 74.7, 73.4, 72.2, 68.8, -3.6; mass spectrum (CI) *m/z* 707.2591 [$C_{42}H_{44}O_6SiCl$ (M+1) requires 707.2596], 601, 493, 391 (base), 265.

NMR assignments. ^{1}H NMR (300 MHz) δ 7.40-6.98 (comp, 20 H, aromatic H), 6.67 (s, 1 H, C13-H), 6.21 (dd, *J* = 19.6, 14.6 Hz, 1 H, C16-H), 6.06 (dd, *J* = 14.6, 4.3 Hz, 1 H, C17-H), 5.81 (d, *J* = 19.6, 4.3 Hz, 1 H, C18-H), 5.00-4.85 (comp, 3 H, benzylic H), 4.72-4.48 (comp, 5 H, benzylic H), 4.10 (d, *J* = 10.5 Hz, 1 H, C1-H), 4.12-4.00 (m, 1 H, C2-H), 3.88-3.70 (comp, 4 H, C3-H & C4-H & C6-H), 3.70-3.58 (m, 1 H, C5-H) 0.35 (s, 6 H, C15-H)



4.69

2-[4-Chloro-5-(3,4,5-trisbenzyloxy-6-benzyloxymethyltetrahydropyran-2-yl)-furan-2-yl]-dimethylsilanylethanol (4.69). By applying similar method to make **4.66,** alcohol **4.69** was prepared from vinylsilane **4.68** (118 mg, 0.16 mmol), 9-BBN (0.36 mL, 0.18 mL), 30% H_2O_2 (0.12 mL) and 3 N NaOH (0.12 mL) as a clear liquid (67%, 80 mg): ^{1}H NMR (300 MHz) δ 7.42-7.20 (comp, 18 H), 7.08-7.00 (comp, 2 H), 6.68 (s, 1 H), 5.01-4.85 (comp, 3 H), 4.74-4.50 (comp, 5 H), 4.11 (d, *J* = 10.5 Hz, 1 H), 4.12-4.02 (m, 1 H), 3.90-3.60 (comp, 5 H), 3.75 (t, *J* = 8.2 Hz, 2 H), 1.40-1.28 (br s), 1.18 (d, *J* = 8.2 Hz, 2 H), 0.32 (s, 3 H), 0.31 (s, 3 H); mass spectrum (CI) *m/z* 727.2846 [$C_{42}H_{48}O_7Si_1Cl$ (M+1) requires 727.2858] (base), 517.

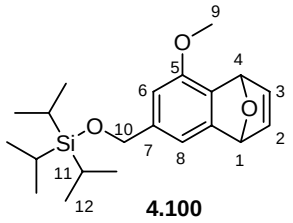


4.70

3,4,5-Tris(benzyloxy)-2-benzyloxymethyl-6-[5-({2-[3-(*tert*-butyldimethylsilanyloxymethyl)-5-chlorophenoxy]-ethyl}-dimethylsilanyl)-3-chloro-furan-2-yl]-tetrahydropyran (4.70). **(3-146).** DIAD (40.4 mg, 0.2 mmol) was added to a solution of alcohol **4.69** (96 mg, 0.13 mmol), phenol **4.29** (54 mg, 0.2 mmol) and triphenylphosphine (52 mg, 0.4 mmol) in benzene (1 mL). The mixture was stirred at rt for 15 min. Solvents evaporated under reduced pressure and the residue was purified by chromatography (Et_3N deactivated silica) eluting with Et_2O/hexanes (1:10) to yield 40

mg (31%) of **4.70** as a clear liquid: ^{1}H NMR (300 MHz, benzene-d_6) δ 7.36-6.96 (comp, 21 H), 6.86 (m, 1 H), 6.80 (m, 1 H), 6.38 (s, 1 H), 4.96-4.82 (comp, 3 H), 4.69 (d, J = 9.3 Hz, 1 H), 4.72-4.60 (comp, 2 H), 4.45 (d, J = 12.0 Hz, 1 H), 4.42 (s, 2 H), 4.32-4.24 (comp, 2 H), 4.21 (app t, J = 9.9, 9.0 Hz, 1 H), 3.93 (t, J = 9.3 Hz , 1 H), 3.78-3.54 (comp, 5 H), 3.48-3.36 (m, 1 H), 1.00 (t, J = 7.4 Hz, 2 H), 0.95 (s, 9 H), 0.07 (s, 6 H), 0.00 (s, 6 H); ^{13}C NMR (75 MHz, benzene-d_6) δ 160.1, 159.2, 151.7, 146.7, 145.1, 139.5, 139.3, 139.1, 138.8, 135.2, 122.7, 120.2, 118.7, 116.1, 113.2, 111.3, 87.0, 81.0, 80.2, 78.3, 75.6, 75.0, 74.8, 73.6, 72.9, 69.2, 64.9, 64.3, 26.0, 18.5, 15.8, -3.3, -5.3; IR (benzene) 2927, 2856, 1577, 1453 cm^{-1}; mass spectrum (CI) *m/z* 980.3650 [$C_{55}H_{65}O_8Si_2Cl_2$ (M) requires 980.3673], 849 (base), 431.

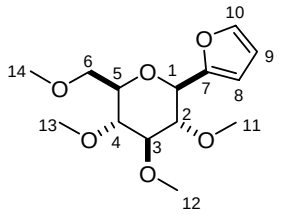
NMR assignments. ^{1}H NMR (300 MHz, benzene-d_6) δ 7.36-6.96 (comp, 21 H, aromatic-H), 6.86 (m, 1 H, aromatic-H), 6.80 (m, 1 H, aromatic-H), 6.38 (s, 1 H, C12-H), 4.96-4.82 (comp, 3 H, benzylic-H), 4.69 (d, J = 9.3 Hz, 1 H, C1-H), 4.72-4.60 (comp, 2 H, benzylic-H), 4.45 (d, J = 12.0 Hz, 1 H, benzylic-H), 4.42 (s, 2 H, C23-H), 4.32-4.24 (comp, 2 H, benzylic-H), 4.21 (app t, J = 9.9, 9.0 Hz, 1 H, C2-H), 3.93 (t, J = 9.3 Hz, 1 H, C4-H), 3.78-3.54 (comp, 5 H, C3-H & C6-H & C16-H), 3.48-3.36 (m, 1 H, C5-H), 1.00 (t, J = 7.4 Hz, 2 H, C15-H), 0.95 (s, 9 H, C25-H), 0.07 (s, 6 H, C27-H), 0.00 (s, 6 H, C24-H); ^{13}C NMR (75 MHz, benzene-d_6) δ 160.1, 159.2 (C11), 151.7 (C14), 146.7, 145.1, 139.5, 139.3, 139.1, 138.8, 135.2, 122.7 (C12), 120.2, 118.7, 116.1 (C13), 113.2, 111.3, 87.0 (C3), 81.0 (C2), 80.2 (C5), 78.3 (C4), 75.6, 75.0, 74.8, 73.6 (benzylic-C), 72.9 (C1), 69.2 (C6), 64.9 (C23), 64.3 (C16), 26.0 (C25), 18.5 (C26), 15.8 (C15), -3.3 (C27), -5.3 (C24).



4.100

Triisopropyl-(6-methoxy-11-oxatricyclo[6.2.1.0^{2,7}]undeca-2,4,6,9-tetraen-4-ylmethoxysilane (4.100). (3-256AB). A solution of *sec*-BuLi (1.16 M, 270 μL, 0.313 mmol) in cyclohexane was added dropwise to a stirred solution of **4.39b** (100 mg, 0.304 mmol) in THF (2 mL) at –95 °C. The reaction was stirred for 25 min at –95 °C, and then furan (220 μL) was added dropwise. The reaction was allowed to warm up slowly to 0 °C whereupon saturated NH_4Cl/H_2O (3 mL, 5:1) was added. The mixture was extracted with EtOAc (3 x 6 mL). The combined organic layers were dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by flash chromatography eluting with Et_2O/Hexanes (1:7) to afford 72 mg (66%) of **4.100** as a clear oil: ^{1}H NMR (400 MHz) δ 7.03 (dd, *J* = 5.5, 1.7 Hz, 1 H), 6.98 (dd, *J* = 5.5, 1.7 Hz, 1 H), 6.87 (s, 1 H), 6.65 (s, 1 H), 5.90 (s, 1 H), 5.65 (s, 1 H), 4.75 (s, 2 H), 3.80 (s, 3 H), 1.30-1.10 (comp, 21 H); ^{13}C NMR (100 MHz) δ 152.1, 151.0, 142.6, 142.2, 140.9, 132.9, 111.0, 107.3, 82.5, 80.0, 64.9, 55.8, 18.5, 12.5; mass spectrum (CI) *m/z* 361.2194 [$C_{21}H_{33}O_3Si$ (M+1) requires 361.2199], 317, 187 (base).

NMR assignments. ^{1}H NMR (400 MHz) δ 7.03 (dd, *J* = 5.5, 1.7 Hz, 1 H), 6.98 (dd, *J* = 5.5, 1.7 Hz, 1 H, C2-H), 6.87 (s, 1 H, C8-H), 6.65 (s, 1 H, C6-H), 5.90 (s, 1 H, C4-H), 5.65 (s, 1 H, C1-H), 4.75 (s, 2 H, C10-H), 3.80 (s, 3 H, C9-H), 1.30-1.10 (comp, 21 H, C11-H & C12-H).



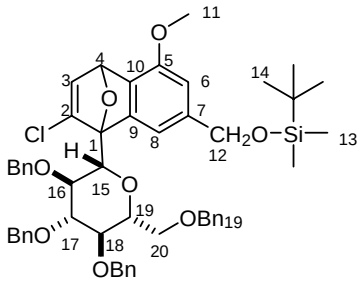
4.113

(2*R*,3*R*,4*S*,5*R*,6*R*)-2-(furan-2-yl)-tetrahydro-3,4,5-trimethoxy-6-(methoxymethyl)-2H-pyran (4.113). (3-234). To a solution of the acetyl-2,3,4,6-*tetra*-O-methyl-D-glucopyranose **4.112** (162 mg, 2.80 mmol) and furan (5.7 mL, 80 mmol) in CH_2Cl_2 (25 mL) was added $BF_3.Et_2O$ (0.28 mL, 2.28 mmol) and the solution was stirred at rt overnight. The mixtures were poured into $NaHCO_3$ (30 mL) and diluted with CH_2Cl_2 (30 mL), and the layers were separated. The organic layer was washed with $NaHCO_3$ (30 mL), dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by flash chromatography, eluting with EtOAc/hexanes (1:3) to yield 475 mg (61%) **4.113** as a colorless oil: ^{1}H NMR (400 MHz) δ 7.40 (dd, *J* = 1.0, 0.7 Hz, 1 H), 6.39 (d, *J* = 3.1 Hz, 1 H), 6.35 (dd, *J* = 3.1, 1.8 Hz, 1 H), 4.15 (d, *J* = 9.9 Hz, 1 H), 3.66 (s, 3 H), 3.63 (dd, *J* = 10.6, 2.1 Hz, 1 H), 3.60-3.54 (m, 1 H), 3.57 (s, 3 H), 3.48-3.36 (comp, 2 H), 3.37 (s, 3 H), 3.27 (t, *J* = 8.5 Hz, 1 H), 3.22 (t, *J* = 9.0 Hz, 1 H), 3.17 (s, 3 H); ^{13}C NMR (75 MHz) δ 151.1, 142.4, 110.4, 109.8, 88.3, 82.4, 79.6, 79.0, 74.5, 71.5, 60.8, 60.5, 59.9, 59.3; mass spectrum (CI) *m/z* 287.1497 [$C_{14}H_{23}O_6$ (M+1) requires 287.1495] 255, 223, 163 (base), 145.

NMR assignments. ^{1}H NMR (400 MHz) δ 7.40 (dd, *J* = 1.0, 0.7 Hz, 1 H, C10-H), 6.39 (d, *J* = 3.1 Hz, 1 H, C9-H), 6.35 (dd, *J* = 3.1, 1.8 Hz, 1 H, C8-H), 4.15 (d, *J* = 9.9 Hz, 1 H, C1-H), 3.66 (s, 3 H, Sugar-OCH_3), 3.63 (dd, *J* = 10.6, 2.1 Hz, 1 H, C6-H), 3.60-3.54 (m, 1 H, C6-H), 3.57 (s, 3 H, Sugar-OCH_3), 3.48-3.36 (comp, 2 H, C5-H & C3-H), 3.37 (s, 3 H, Sugar-OCH_3), 3.27 (t, *J* = 8.5 Hz, 1 H), 3.22 (t, *J* = 9.0 Hz, 1 H), 3.17 (s, 3

H); ^{13}C NMR (75 MHz) δ 151.1 (C7), 142.4 (C10), 110.4 (C9), 109.8 (C8), 88.3 (C2), 82.4 (C1), 79.6 , 79.0, 74.5 (C6), 71.5 (C5), 60.8 (C13), 60.5 (C11), 59.9 (C14), 59.3 (C12).

General procedure for glucosyl furan Diels-Alder reactions. *sec*-Butyllithium (0.25 mL, 0.29 mmol, 1.16 M in cyclohexanes) was added dropwise to a solution of 2-chloro-1,4-dimethoxybenzene (**4.103**, 50 mg, 0.29 mmol), in THF (1.5 mL) at -95 °C. The mixture was stirred for 15~25 min and a solution of glycosyl furan (0.088 mmol) in THF (0.6 mL) was added at -95 °C. The reaction was warmed -95 °C without removing cooling bath whereupon, NH_4Cl (3 mL) and H_2O (0.5 mL) were added and poured onto H_2O (20 mL), and the resulting mixture was extracted with EtOAc (2 x 15 mL). The mixture was extracted with EtOAc (3 x 5 mL). The combined organic layers were dried (Na_2SO_4), filtered and concentrated, and the residue was purified by flash chromatography, eluting with Et_2O/hexanes or EtOAc/hexanes. (Note: Those cycloadduct, often isolated as a mixture of diastereomers were typically not fully characterized, only partial spectral data were available.)

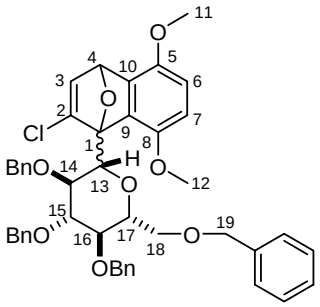


4.95a

Cycloadduct (4.95a). Cycloadduct **4.95a** was prepared in 25% yield (single diastereomer) as a pale clear oil according to the general procedure described above: ^{1}H

NMR (400 MHz) δ 7.42-6.98 (comp, 23 H), 6.36 (s, 1 H), 5.04-4.82 (comp, 3 H), 4.80-4.28 (comp, 6 H), 4.60 (s, 2 H), 4.04-3.96 (m, 1 H), 3.88-3.62 (comp, 3 H), 3.58-3.48 (m, 1 H), 3.39 (s, 3 H), 3.44-3.22 (m, 1 H), 1.06 (s, 9 H), 0.11 (s, 3 H); mass spectrum (CI) *m/z* 875.3734 [$C_{52}H_{60}O_8SiCl$ (M+1) requires 875.3746], 840, 767, 659 (base).

NMR assignments. ^{1}H NMR (400 MHz) δ 7.42-6.98 (comp, 23 H, aromatic-H & C3-H &C6-H &C8-H), 6.36 (s, 1 H, C4-H), 5.04-4.82 (comp, 3 H, benzylic-H), 4.80-4.28 (comp, 6 H, C1-H & benzylic-H), 4.60 (s, 2 H, C12-H), 4.04-3.96 (m, 1 H, C16-H), 3.88-3.62 (comp, 3 H, C17-H & C20-H), 3.58-3.48 (m, 1 H, C19-H), 3.39 (s, 3 H, OMe-H), 3.44-3.22 (m, 1 H, C18-H), 1.06 (s, 9 H, C14-H), 0.11 (s, 3 H, C13-H).



4.114

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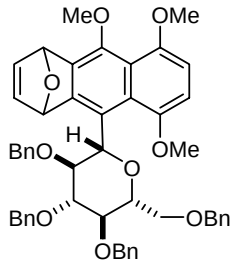
Cycloadduct (4.114). (3-288) Cycloadduct **4.114** was prepared in 76% yield (8:1 mixture of diastereomers) as pale yellow oil according to the general procedure described above: ^{1}H NMR (400 MHz) δ 7.42-7.14 (comp, 20 H), 6.69 (d, *J* = 1.7 Hz, 0.89 H, major isomer), 6.65 (d, *J* = 1.7 Hz, 0.11 H, minor isomer), 6.63 (d, *J* = 8.9 Hz, 0.89 H, major isomer), 6.58 (s, 0.22 H, minor isomer), 6.52 (d, *J* = 8.9 Hz, 0.89 H, major isomer), 6.95 (d, *J* =1.7 Hz, 0.89 H, major isomer), 6.01 (d, *J* = 1.7 Hz, 0.11 H, minor isomer), 5.06-4.78 (comp, 5 H), 4.65 (d, *J* = 10.6 Hz, 1 H), 4.53-4.40 (comp, 3 H), 4.00-3.60 (comp, 6 H), 3.82 (s, 3 H), 3.64 (s, 3 H). ^{13}C NMR (100 MHz) δ 149.8, 148.9, 147.5, 138.6, 138.4, 138.3, 138.2, 137.7, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 125.5, 127.4,

127.3, 127.2, 112.1, 114.5, 94.4, 87.5, 80.1, 79.8, 79.1, 78.9, 75.7, 75.1, 74.9, 72.9, 69.1, 56.3, 55.9.

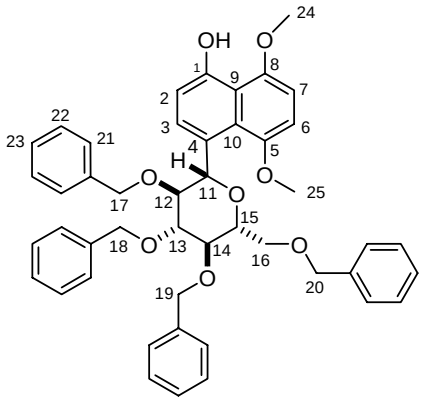
NMR assignments. ^{1}H NMR (400 MHz) δ 7.42-7.14 (comp, 20 H, aromatic-H), 6.69 (d, *J* = 1.7 Hz, 0.89 H, major isomer, C3-H), 6.65 (d, *J* = 1.7 Hz, 0.11 H, minor isomer, C3-H), 6.63 (d, *J* = 8.9 Hz, 0.89 H, major isomer, C6-H), 6.58 (s, 0.22 H, minor isomer, C6-H & C7-H), 6.52 (d, *J* = 8.9 Hz, 0.89 H, major isomer, C7-H), 6.95 (d, *J* =1.7 Hz, 0.89 H, major isomer, C4-H), 6.01 (d, *J* = 1.7 Hz, 0.11 H, minor isomer, C4-H), 5.06-4.78 (comp, 5 H, benzylic-H & C1-H), 4.65 (d, *J* = 10.6 Hz, 1 H, benzylic-H), 4.53-4.40 (comp, 3 H, benzylic-H), 4.00-3.60 (comp, 6 H, C14-H & C15-H & C16-H & C17-H & C18-H), 3.82 (s, 3 H, C12-H), 3.64 (s, 3 H, C11-H).



4.143

Cycloadduct (4.143). (4-227). According to the general procedure described above, cycloadduct **4.143** was obtained in 55% yield as a thick oil from the cycloaddition of chloronaphthalene **4.142** (23 mg, 0.030 mmol) and furan (25 μL, 0.30 mmol) after purification by flash chromatography eluting with Et_2O/Hexanes (1:2): ^{1}H NMR (400 MHz) δ 7.38-7.16 (comp, H), 7.08-6.72 (comp, 22 H), 6.2 (app d. *J* = 6.8 Hz, 1 H), 6.40 (d, *J* = 9.6 Hz, 1 H), 6.37 (d, *J* = 7.2 Hz, 1 H), 6.28-6.23 (m, 1 H), 6.01 (br s, 1 H), 5.02-4.80 (comp, 3 H), 4.68-4.44 (comp, 3 H), 4.29 (d, *J* = 10.6 Hz, 0.5 H), 4.09 (d, *J* = 9.9 Hz, 0.5 H), 4.00-3.58 (comp, 6 H), 3.27 (d, *J* = 10.3 Hz, 0.5 H), 3.13 (t, *J* = 9.2 Hz, 0.5

H); IR (CH_2Cl_2) 3029, 2864, 1668, 1565, 1453, 1094 cm^{-1}; MS (CI) *m/z* 807.3536 [$C_{51}H_{51}O_9$ (M+1) requires 807.3533] (base).



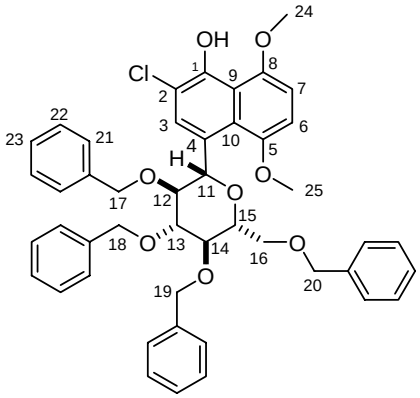
4.133

5,8-Dimethoxy-4-(3,4,5-tris-benzyloxy-6-benzyloxymethyltetrahydropyran-2-yl)-naphthalen-1-ol (4.133). (4-243, 4-244). According to the general procedure described above, crude cycloadduct **4.132** (2.57 g) was obtained as a thick oil along with recovered **4.104** (386 mg) from glucosylfuran **4.104** (1.97 g, 3.34 mmol) and 2-chloro-1,4-dimethoxy-benzne (1.72 g, 10.0 mmol) after purification by flash chromatography eluting with Et_2O/hexanes (1:2).

$BF_3{\cdot}Et_2O$ (960 μL, 7.58 mmol) was added to a solution of the crude cycloadduct (2.57 g, 3.54 mmol) obtained above dissolved in CH_2Cl_2 (75 mL) containing 2,6-lutidine (600 μL, 5.15 mmol) at -5°C. The mixture was stirred at -5°C overnight, and more $BF_3{\cdot}Et_2O$ (220 μL, 1.58 mmol) was added. The reaction was stirred for another 6 h, whereupon saturated $NaHCO_3$ (50 mL) was added. The layers were separated and the aqueous layer was extracted with EtOAc (2 x 40 mL). The combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by flash chromatography eluting with Et_2O/hexanes (1:2) to afford 1.477 g (61%, 76% brsm over two steps) of **4.133** as a thick oil: ^1H NMR (400 MHz) δ 10.03 (s, 1 H), 7.86 (d, *J* =

8.2 Hz, 1 H), 7.48-7.28 (comp, 15 H), 7.16 (tt, *J* = 7.2, 1.4 Hz, 1 H), 7.08 (app t, *J* = 7.2 Hz, 2 H), 7.04 (d, *J* = 8.2 Hz, 1 H), 6.74 (s, 2 H), 6.64 (d, *J* = 8.2 Hz, 2 H), 6.24 (d, *J* = 9.2 Hz, 1 H), 5.05 (d, *J* = 10.9 Hz, 1 H), 4.99 (d, *J* = 10.6 Hz, 1 H), 4.97 (d, *J* = 10.9 Hz, 1 H), 4.76 (d, *J* = 12.3 Hz, 1 H), 4.74 (d, *J* = 10.6 Hz, 1 H), 4.65 (d, *J* = 12.3 Hz, 1 H), 4.35 (d, *J* = 10.3 Hz, 1 H), 4.03 (s, 3 H), 4.00-3.76 (comp, 5 H), 3.83 (s, 3 H), 3.72 (d, *J* = 10.3 Hz, 1 H), 3.63 (t, *J* = 8.9 Hz, 1 H); ^{13}C NMR (100 MHz) δ 154.6, 152.6, 150.4, 138.8, 138.4, 138.3, 138.0, 128.32, 128.26, 128.2, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.3, 127.1, 127.01, 127.00, 116.2, 111.4, 106.4, 103.6, 87.1, 86.0, 79.4, 78.8, 75.4, 75.0, 74.4, 73.3, 69.3, 56.4, 56.1; IR (CH_2Cl_2) 3354, 3029, 2883, 1619, 1526, 1453, 1060 cm^{-1}; MS (CI) *m/z* 726.3193 [$C_{46}H_{46}O_8$ (M) requires 726.3193], 619, 511 (base).

NMR assignments. ^{1}H NMR (400 MHz) δ 10.03 (s, 1 H, OH), 7.86 (d, *J* = 8.2 Hz, 1 H, C3-H), 7.48-7.28 (comp, 15 H), 7.16 (tt, *J* = 7.2, 1.4 Hz, 1 H, C23-H), 7.08 (app t, *J* = 7.2 Hz, 2 H, C22-H), 7.04 (d, *J* = 8.2 Hz, 1 H, C2-H), 6.74 (s, 2 H, C6-H & C7-H), 6.64 (d, *J* = 8.2 Hz, 2 H, C21-H), 6.24 (d, *J* = 9.2 Hz, 1 H. C11-H), 5.05 (d, *J* = 10.9 Hz, 1 H, C18-H), 4.99 (d, *J* = 10.6 Hz, 1 H, C19-H), 4.97 (d, *J* = 10.9 Hz, 1 H, C18-H), 4.76 (d, *J* = 12.3 Hz, 1 H, C20-H), 4.74 (d, *J* = 10.6 Hz, 1 H, C19-H), 4.65 (d, *J* = 12.3 Hz, 1 H, C20-H), 4.35 (d, *J* = 10.3 Hz, 1 H, C17-H), 4.03 (s, 3 H, C24-H), 4.00-3.76 (comp, 5 H, C13-H & C14-H &C15-H & C16-H), 3.83 (s, 3 H, C25-H), 3.72 (d, *J* = 10.3 Hz, 1 H, C17-H), 3.63 (t, *J* = 8.9 Hz, 1 H, C12-H); ^{13}C NMR (100 MHz) δ 154.6, 152.6, 150.4, 138.8, 138.4, 138.3, 138.0, 128.32, 128.26, 128.2, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.3, 127.1, 127.01, 127.00, 116.2, 111.4, 106.4, 103.6, 87.1 (C13), 86.0 (C12), 79.4 (C14), 78.8 (C15), 75.4 (C18), 75.0 (C19), 74.4 (C17), 73.3 (C20), 69.3 (C16), 56.4 (C24), 56.1 (C25).

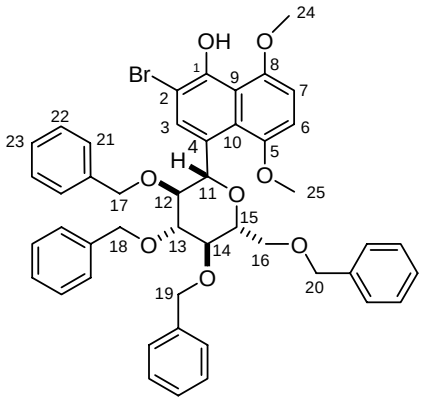


4.141

2-Chloro-5,8-dimethoxy-4-(3,4,5-tris(benzyloxy)-6-benzyloxymethyl-tetrahydropyran-2-yl)-naphthalen-1-ol (4.141). **(4-196).** A solution of naphthol **4.133** (51 mg, 0.070 mmol) and NCS (10.3 mg, 0.077 mmol) in AcOH (1.5 mL) was stirred at rt for 2 d. The solvent was evaporated under reduced pressure. The residue was purified by flash chromatography eluting with Et_2O/hexanes (1:3 then 1:2) to afford 20 mg (37%) of **4.141** as a light green oil: ^{1}H NMR (400 MHz) δ 10.55 (s, 1 H), 7.82 (s, 1 H), 7.40-7.18 (comp, 18 H), 7.09 (app t, *J* = 7.3, 1 H), 7.00 (app t, *J* = 7.5 Hz, 2 H), 6.75 (d, *J* = 8.5 Hz, 1 H), 6.67 (d, *J* = 8.9 Hz, 1 H), 6.53 (d, *J* = 8.2 Hz, 2 H), 6.09 (d, *J* = 9.2 Hz, 1 H), 4.96 (d, *J* = 10.9 Hz, 1 H), 4.93–4.85 (comp, 2 H), 4.68 (d, *J* = 12.3 Hz, 1 H), 4.64 (d, *J* = 10.6 Hz, 1 H), 4.60 (d, *J* = 12.3 Hz, 1 H), 4.33 (d, *J* = 10.4 Hz, 1 H), 4.05 (s, 3 H), 3.90-3.66 (comp, 5 H), 3.76 (s, 3 H), 3.69 (d, *J* = 10.4 Hz, 1 H), 3.50-3.44 (m, 1 H); ^{13}C NMR (100 MHz) δ 152.8, 149.8, 149.6, 138.8, 138.4, 138.2, 137.9, 128.6, 128.42, 128.36, 128.2, 128.1, 127.9, 127.73, 127.70, 127.6, 127.50, 127.47, 127.2, 125.6, 116.9, 116.3, 109.7, 106.6, 105.1, 87.1, 86.0, 79.4, 78.9, 76.7, 75.4, 75.1, 74.7, 73.3, 69.3, 57.0, 56.1; IR (CH_2Cl_2) 3301, 2861, 1618, 1250, 1057, 738, 697 cm^{-1}.

NMR assignments. ^{1}H NMR (400 MHz) δ 10.55 (s, 1 H, OH), 7.82 (s, 1 H, C3-H), 7.40-7.18 (comp, 18 H, benzyl aromatics), 7.09 (app t, *J* = 7.3, 1 H, C23-H), 7.00

(app t, J = 7.5 Hz, 2 H, C22-H), 6.75 (d, J = 8.5 Hz, 1 H, C7-H), 6.67 (d, J = 8.9 Hz, 1 H, C6-H), 6.53 (d, J = 8.2 Hz, 2 H, C21-H), 6.09 (d, J = 9.2 Hz, 1 H, C11-H), 4.96 (d, J = 10.9 Hz, 1 H, C18-H), 4.93–4.85 (comp, 2 H, C18-H & C19-H), 4.68 (d, J = 12.3 Hz, 1 H, C20-H), 4.64 (d, J = 10.6 Hz, 1 H, C19-H), 4.60 (d, J = 12.3 Hz, 1 H, C20-H), 4.33 (d, J = 10.4 Hz, 1 H, C17-H), 4.05 (s, 3 H, C24-H), 3.90-3.66 (comp, 5 H, C13-H & C14-H & C15-H & C16-H), 3.76 (s, 3 H, C25-H), 3.69 (d, J = 10.4 Hz, 1 H, C17-H), 3.50-3.44 (m, 1 H, C12-H); ^{13}C NMR (100 MHz) δ 152.8, 149.8, 149.6, 138.8, 138.4, 138.2, 137.9, 128.6, 128.42, 128.36, 128.2, 128.1, 127.9, 127.73, 127.70, 127.6, 127.50, 127.47, 127.2, 125.6, 116.9, 116.3, 109.7, 106.6, 105.1, 87.1 (C13), 86.0 (C12), 79.4 (C14), 78.9 (C15), 76.7 (C11), 75.4 (C18), 75.1 (C19), 74.7 (C17), 73.3 (C20), 69.3 (C16), 57.0 (C24), 56.1 (C25).



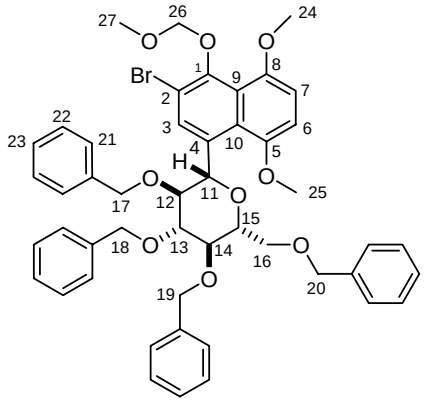
4.138

2-Bromo-5,8-dimethoxy-4-(3,4,5-tris(benzyloxy)-6-benzyloxymethyltetrahydropyran-2-yl)naphthalen-1-ol (4.138). (4-274). A solution of Br_2 (0.15 mL, 2.92 mmol) in CCl_4 (2 mL) was added to naphthol **4.133** (1.86 g, 2.56 mmol) at 0 °C in CCl_4 (24 mL). The mixture was stirred at 0 °C for 3 min. The reaction was quenched by addition of saturated $NaHCO_3$ (40 mL). The layers were separated, and the aqueous layer was extracted with $CHCl_3$ (2 x 30 mL). The combined organic layers were dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified

by flash chromatography eluting with Et_2O/hexanes (1:2) to afford 1.42 g (69%) of **4.138** as a thick oil: ^{1}H NMR (500 MHz) δ 10.67 (s, 1 H), 7.95 (s, 1 H), 7.39-7.19 (comp, 15 H), 7.08 (tt, *J* = 7.4, 1.3 Hz, 1 H), 7.00 (app t, *J* = 7.3 Hz, 2 H), 6.74 (d, *J* = 8.7 Hz, 1 H), 6.67 (d, *J* = 8.7 Hz, 1 H), 6.53 (app d, *J* = 6.9 Hz, 2 H), 6.07 (d, *J* = 9.3 Hz, 1 H), 4.96 (d, *J* = 11.3 Hz, 1 H), 4.89 (d, *J* = 11.3 Hz, 1 H), 4.88 (d, *J* = 10.7 Hz, 1 H), 4.70-4.62 (comp, 2 H), 4.60 (d, *J* = 12.3 Hz, 1 H), 4.33 (d, *J* = 10.6 Hz, 1 H), 4.02 (s, 3 H), 3.90-3.67 (comp, 5 H), 3.75 (s, 3 H), 3.70 (d, *J* = 10.6 Hz, 1 H), 3.47 (app t, *J* = 9.0 Hz, 1 H); ^{13}C NMR (125 MHz) δ 152.9, 150.6, 149.6, 138.9, 138.4, 138.3, 137.9, 131.2, 128.5, 128.4, 128.35, 128.32, 128.0, 127.9, 127.71, 127.66, 127.56, 127.5, 127.4, 127.1, 126.1, 116.8, 106.8, 105.7, 105.2, 87.2, 86.0, 79.5, 78.9, 76.7, 75.3, 75.1, 74.7, 73.3, 69.3, 57.0, 56.2; IR (CH_2Cl_2) 3299, 2861, 1616, 1452, 1249, 1097, 1058 cm^{-1}; MS (CI) *m/z* 804.2289 [$C_{46}H_{45}O_8Br$ (M) requires 804.2298]; (CI-) 806 (base), 804, 762, 671.

NMR assignments. ^{1}H NMR (500 MHz) δ 10.67 (s, 1 H, OH), 7.95 (s, 1 H, C3-H), 7.39-7.19 (comp, 15 H, benzyl aromatics), 7.08 (tt, *J* = 7.4, 1.3 Hz, 1 H, C23-H), 7.00 (app t, *J* = 7.3 Hz, 2 H, C22-H), 6.74 (d, *J* = 8.7 Hz, 1 H, C7-H), 6.67 (d, *J* = 8.7 Hz, 1 H, C6-H), 6.53 (d, *J* = 6.9 Hz, 2 H, C21-H), 6.07 (d, *J* = 9.3 Hz, 1 H, C11-H), 4.96 (d, *J* = 11.3 Hz, 1 H, C18-H), 4.89 (d, *J* = 11.3 Hz, 1 H, C18-H), 4.88 (d, *J* = 10.7 Hz, 1 H, C19-H), 4.70-4.62 (comp, 2 H, C19-H & C20-H), 4.60 (d, *J* = 12.3 Hz, 1 H, C20-H), 4.33 (d, *J* = 10.6 Hz, 1 H, C17-H), 4.02 (s, 3 H, C24-H), 3.90-3.67 (comp, 5 H, C13-H & C14-H & C15-H & C16-H), 3.75 (s, 3 H, C25-H), 3.70 (d, *J* = 10.6 Hz, 1 H, C17-H), 3.47 (app t, *J* = 9.0 Hz, 1 H, C12-H); ^{13}C NMR (125 MHz) δ 152.9, 150.6, 149.6, 138.9, 138.4, 138.3, 137.9, 131.2 (C3), 128.5, 128.4, 128.35, 128.32, 128.0, 127.9, 127.71, 127.66, 127.56, 127.5, 127.4, 127.1, 126.1(C23), 116.8, 106.8 (C6), 105.7, 105.2 (C7), 87.2 (C13), 86.0 (C12), 79.5 (C14), 78.9 (C15), 76.7 (C11), 75.3 (C18), 75.1 (C19), 74.7(C17), 73.3 (C20), 69.3 (C16), 57.0 (C24), 56.2 (C25). Note: ^{1}H-^{13}C HSQC revealed

that the anomeric carbon, as in several other cases, was buried underneath $CDCl_3$ peak (76.7 ppm)

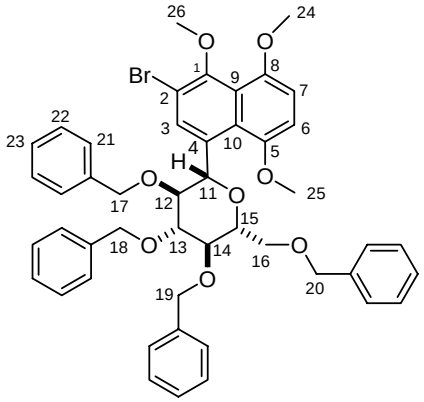


4.139

3,4,5-Tris-benzyloxy-2-benzyloxymethyl-6-(3-bromo-5,8-dimethoxy-4-methoxymethoxynaphthalen-1-yl)-tetrahydropyran (4.139). (**4-195**). To a solution of bromonaphthol **4.138** (85 mg, 0.105 mmol) in DMF (4.5 mL) at 0 °C, was added NaH (6.6 mg, 0.17 mmol, 60% suspension in mineral oil) and then MOMCl (13 μL, 0.17 mmol). The mixture was stirred at rt overnight. Saturated $NaHCO_3$ (15 mL) was added, and the mixture was extracted with EtOAc (3 x 15 mL). The combined organic layers were washed with NaCl (15 mL), dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by flash chromatography eluting with Et_2O/hexanes (1:2 then 2:3) to afford 85 mg (95%) of **4.139** as a thick oil: ^{1}H NMR (500 MHz) δ 8.01 (s, 1 H), 7.40-7.18 (comp, 15 H), 7.07 (tt, *J* = 7.4, 1.3 Hz, 1 H), 6.98 (app t, *J* = 7.5 Hz, 2 H), 6.81 (d, *J* = 8.7 Hz, 1 H), 6.72 (d, *J* = 8.7 Hz, 1 H), 6.47 (d, *J* = 8.2 Hz, 12 H), 6.14 (d, *J* = 9.2 Hz, 1 H), 5.10 (d, *J* = 5.4 Hz, 1 H), 5.04 (d, *J* = 5.4 Hz, 1 H), 4.97 (d, *J* = 11.2 Hz, 1 H), 4.90 (d, *J* = 11.2 Hz, 1 H), 4.88 (d, *J* = 10.8 Hz, 1 H), 4.67 (d, *J* = 12.4 Hz, 1 H), 4.65 (d, *J* = 10.8 Hz, 1 H), 4.60 (d, *J* = 12.4 Hz, 1 H), 4.32 (d, *J* = 10.4 Hz, 1 H), 3.90 (s, 3 H), 3.88-3.70 (comp, 5 H), 3.75 (s, 3 H), 3.71 (s, 3 H), 3.62 (d, *J* = 10.4 Hz, 1 H),

3.47 (app t , J = 9.0 Hz, 1 H);); ^{13}C NMR (100 MHz) δ 152.3, 149.4, 149.1, 138.7, 138.3, 138.1, 137.6, 134.1, 130.2, 128.4, 128.3, 128.0, 127.9, 127.7, 127.6, 127.5, 127.4, 127.2, 126.9, 122.5, 116.0, 107.7, 100.8, 87.1, 85.9, 79.5, 78.7, 75.3, 75.1, 74.7, 73.3, 69.1, 58.3, 57.0, 56.2; IR (CH_2Cl_2) 2901, 1608, 1570, 1453, 1258, 1059 cm^{-1}; MS (CI) *m/z* 848.2556 [$C_{48}H_{49}O_9Br$ (M) requires 848.2460], 849 (base), 817, 741, 645.

NMR assignments. ^{1}H NMR (500 MHz) δ 8.01 (s, 1 H, C3-H), 7.40-7.18 (comp, 15 H, benzyl aromatics), 7.07 (tt, J = 7.4, 1.3 Hz, 1 H, C23-H), 6.98 (app t, J = 7.5 Hz, 2 H, C22-H), 6.81 (d, J = 8.7 Hz, 1 H, C7-H), 6.72 (d, J = 8.7 Hz, 1 H, C6-H), 6.47 (d, J = 8.2 Hz, 2 H, C21-H), 6.14 (d, J = 9.2 Hz, 1 H, C11-H), 5.10 (d, J = 5.4 Hz, 1 H, C26-H), 5.04 (d, J = 5.4 Hz, 1 H, C26-H), 4.97 (d, J = 11.2 Hz, 1 H, C18-H), 4.90 (d, J = 11.2 Hz, 1 H, C18-H), 4.88 (d, J = 10.8 Hz, 1 H, C19-H), 4.67 (d, J = 12.4 Hz, 1 H, C20-H), 4.65 (d, J = 10.8 Hz, 1 H, C19-H), 4.60 (d, J = 12.4 Hz, 1 H, C20-H), 4.32 (d, J = 10.4 Hz, 1 H, C17-H), 3.90 (s, 3 H, C24-H), 3.88-3.70 (comp, 5 H, C13-H & C14-H & C15-H & C16-H), 3.75 (s, 3 H, C25-H), 3.71 (s, 3 H. C27-H), 3.62 (d, J = 10.4 Hz, 1 H, C17-H), 3.47 (app t , J = 9.0 Hz, 1 H, C12-H); ^{13}C NMR (100 MHz) δ 152.3, 149.4, 149.1, 138.7, 138.3, 138.1, 137.6, 134.1, 130.2, 128.4, 128.3, 128.0, 127.9, 127.7, 127.6, 127.5, 127.4, 127.2, 126.9, 122.5, 116.0, 107.7, 100.8 (C26), 87.1 (C13), 85.9 (C12), 79.5 (C14), 78.7(C15), 75.3 (C18), 75.1 (C19), 74.7 (C17), 73.3 (C20), 69.1 (C16), 58.3 (C27), 57.0 (C24), 56.2 (C25).

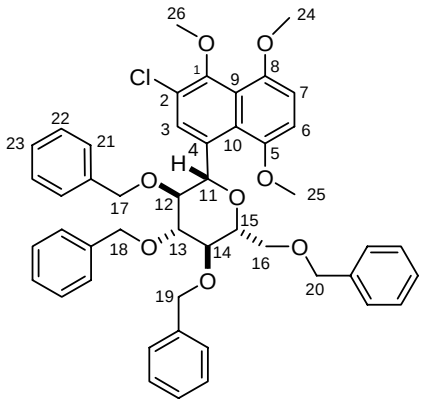


4.140

3,4,5-Tris(benzyloxy)-2-benzyloxymethyl-6-(3-bromo-4,5,8-trimethoxynaphthalen-1-yl)tetrahydropyran (4.140). (4-254). To a solution of bromonaphthol **4.138** (802 mg, 0.998 mmol) in DMF (24 mL) at 0 °C, was added NaH (80 mg, 2.00 mmol, 60% suspension in mineral oil) and then MeI (125 μL, 2.00 mmol). The mixture was stirred at rt overnight. Saturated $NaHCO_3$ (25 mL) and H_2O (10 mL) were added, and the mixture was extracted with EtOAc (2 x 50 mL). The combined organic layers were washed with H_2O (3 x 60 mL), dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by flash chromatography eluting with Et_2O/Hexanes (1:2) to afford 780 mg (96%) of **4.140** as a thick oil: ^{1}H NMR (400 MHz) δ 8.00 (s, 1 H), 7.41-7.18 (comp, 15 H), 7.08 (app t, *J* = 7.4 Hz, 1 H), 6.99 (t, *J* = 7.5 Hz, 2 H), 6.84 (d, *J* = 8.7 Hz, 1 H), 6.75 (d, *J* = 8.7 Hz, 1 H), 6.48 (d, *J* = 7.2 Hz, 1 H), 6.16 (d, *J* = 9.2 Hz, 1 H), 4.97 (d, *J* = 11.1 Hz, 1 H), 4.90 (d, *J* = 11.1 Hz, 1 H), 4.89 (d, *J* = 10.6 Hz, 1 H), 4.68 (d, *J* = 12.3 Hz, 1 H), 4.64 (d, *J* = 10.6 Hz, 1 H), 4.60 (d, *J* = 12.3 Hz, 1 H), 4.33 (d, *J* = 10.6 Hz, 1 H), 3.94 (s, 3 H), 3.90-3.70 (comp, 5 H), 3.86 (s, 3 H), 3.76 (s, 3 H), 3.65 (d, *J* = 10.6 Hz, 1 H), 3.49 (app t , *J* = 9.0 Hz, 1 H); ^{13}C NMR (100 MHz) δ 152.8, 152.3, 149.9, 138.8, 138.4, 138.2, 137.7, 134.0, 130.3, 128.4, 128.1, 128.0, 127.8, 127.7, 127.6, 127.50, 127.48, 127.2, 126.9, 123.0, 115.9, 108.1, 107.9, 87.1, 85.9, 79.5,

78.8, 75.4, 75.1, 74.8, 73.3, 69.2, 61.8, 57.5, 56.3; IR (CH_2Cl_2) 2930, 1608, 1569, 1496, 1259, 1099, 1056 cm^{-1}; MS (CI) *m/z* 818.2456 [$C_{47}H_{47}O_8Br$ (M) requires 818.2454], 605, 519, 445, 433 (base).

NMR assignments. ^1H NMR (400 MHz) δ 8.00 (s, 1 H, C3-H), 7.41-7.18 (comp, 15 H, benzylic aromatics), 7.08 (app t, *J* = 7.4 Hz, 1 H, C23-H), 6.99 (t, *J* = 7.5 Hz, 2 H, C22-H), 6.84 (d, *J* = 8.7 Hz, 1 H, C7-H), 6.75 (d, *J* = 8.7 Hz, 1 H, C6-H), 6.48 (d, *J* = 7.2 Hz, 2 H, C21-H), 6.16 (d, *J* = 9.2 Hz, 1 H, C11-H), 4.97 (d, *J* = 11.1 Hz, 1 H, C18-H), 4.90 (d, *J* = 11.1 Hz, 1 H, C18-H), 4.89 (d, *J* = 10.6 Hz, 1 H, C19-H), 4.68 (d, *J* = 12.3 Hz, 1 H, C20-H), 4.64 (d, *J* = 10.6 Hz, 1 H, C19-H), 4.60 (d, *J* = 12.3 Hz, 1 H, C20-H), 4.33 (d, *J* = 10.6 Hz, 1 H, C17-H), 3.94 (s, 3 H, C24-H), 3.90-3.70 (comp, 5 H, C13-H & C14-H & C15-H & C16-H), 3.86 (s, 3 H, C25-H), 3.76 (s, 3 H, C26-H), 3.65 (d, *J* = 10.6 Hz, 1 H, C17-H), 3.49 (app t , *J* = 9.0 Hz, 1 H, C12-H); ^{13}C NMR (100 MHz) δ 152.8, 152.3, 149.9, 138.8, 138.4, 138.2, 137.7, 134.0, 130.3, 128.4, 128.1, 128.0, 127.8, 127.7, 127.6, 127.50, 127.48, 127.2, 126.9, 123.0, 115.9, 108.1, 107.9, 87.1 (C13), 85.9 (C12), 79.5 (C14), 78.8 (C15), 75.4 (C18), 75.1 (C19), 74.8 (C17), 73.3 (C20), 69.2 (C16), 61.8 (C26), 57.5 (C24), 56.3 (C25).

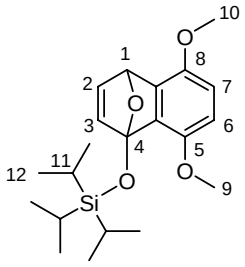


4.142

3,4,5-Tris(benzyloxy)-2-benzyloxymethyl-6-(3-chloro-4,5,8-trimethoxynaphthalen-1-yl)tetrahydropyran (4.142). (4-215). A solution of *n*-BuLi in hexane (90 μL, 2.2 M, 0.198 mmol) was added to bromonaphthalene **4.140** (128 mg, 0.168 mmol) in THF (1.2 mL) at –78°C. The mixture was stirred at -78 °C for 50 min, whereupon a solution of C_2Cl_6 (56 mg, 0236 mmol) in THF (0.2 mL) was added. The mixture was stirred at –78 °C for 3 h and then warmed to rt. The solvent was evaporated under reduced pressure, and the residue was purified by flash chromatography eluting with Et_2O/Hexanes (1:2) to afford 72 mg (79%) of **4.142** as a thick oil: ^{1}H NMR (400 MHz) δ 7.91 (s, 1 H), 7.40-7.22 (comp, 15 H), 7.12 (app t. *J* = 7.4 Hz, 1 H), 7.03 (app t, *J* = 7.5 Hz, 2 H), 6.86 (d, *J* = 8.5 Hz, 1 H), 6.77 (d, *J* = 8.5 Hz, 1 H), 6.52 (d, *J* = 7.2 Hz, 2 H), 6.22 (d, *J* = 9.6 Hz, 1 H), 5.02 (d, *J* = 10.7 Hz, 1 H), 4.95 (d, *J* = 11.3 Hz, 1 H), 4.94 (d, *J* = 10.7 Hz, 1 H), 4.72 (d, *J* = 12.5 Hz, 1 H), 4.69 (d, *J* = 11.3 Hz, 1 H), 4.64 (d, *J* = 12.5 Hz, 1 H), 4.37 (d, *J* = 10.3 Hz, 1 H), 3.98 (s, 3 H), 3.96-3.72 (comp, 5 H), 3.92 (s, 3 H), 3.79 (s, 3 H), 3.69 (d, *J* = 10.3 Hz, 1 H), 3.53 (app t, *J* = 9.1 Hz, 1 H); ^{13}C NMR (100 MHz) δ 152.2, 151.6, 150.0, 138.7, 138.3, 138.2, 137.7, 133.7, 128.4, 128.3, 128.0, 127.9, 127.71, 127.65, 127.56, 127.48, 127.46, 127.2, 126.3, 125.8, 123.0, 108.1, 107.7, 87.1, 85.9, 79.5, 78.7, 75.4, 75.1, 74.7, 73.3, 69.2, 61.7, 57.5, 56.3; IR (CH_2Cl_2) 2931, 2864, 1608, 1576, 1453, 1259, 1057 cm^{-1}; MS (CI) *m/z* 774.2960 [$C_{47}H_{47}O_8Cl$ (M) requires 774.2959], 775 (base), 559, 423.

NMR assignments. δ 7.91 (s, 1 H, C3-H), 7.40-7.22 (comp, 15 H, benzyl aromatics), 7.12 (app t. *J* = 7.4 Hz, 1 H, C23-H), 7.03 (app t, *J* = 7.5 Hz, 2 H, C22-H), 6.86 (d, *J* = 8.5 Hz, 1 H, C7-H), 6.77 (d, *J* = 8.5 Hz, 1 H, C6-H), 6.52 (d, *J* = 7.2 Hz, 2 H, C21-H), 6.22 (d, *J* = 9.6 Hz, 1 H, C11-H), 5.02 (d, *J* = 10.7 Hz, 1 H, C18 H), 4.95 (d, *J* = 11.3 Hz, 1 H, C19-H), 4.94 (d, *J* = 10.7 Hz, 1 H, C18-H), 4.72 (d, *J* = 12.5 Hz, 1 H, C20-H), 4.69 (d, *J* = 11.3 Hz, 1 H, C19-H), 4.64 (d, *J* = 12.5 Hz, 1 H, C20-H), 4.37 (d, *J* =

10.3 Hz, 1 H, C17-H), 3.98 (s, 3 H), 3.96-3.72 (comp, 5 H, C13-H & C14-H & C15-H & C16-H), 3.92 (s, 3 H, C25-H), 3.79 (s, 3 H, C27-H), 3.69 (d, *J* = 10.3 Hz, 1 H, C17-H), 3.53 (app t, *J* = 9.1 Hz, 1 H, C12-H); ^{13}C NMR (100 MHz) δ 152.2, 151.6, 150.0, 138.7, 138.3, 138.2, 137.7, 133.7, 128.4, 128.3, 128.0, 127.9, 127.71, 127.65, 127.56, 127.48, 127.46, 127.2, 126.3, 125.8, 123.0, 108.1, 107.7, 87.1 (C13), 85.9 (C12), 79.5 (C14), 78.7 (C15), 75.4 (C18), 75.1 (C19), 74.7 (C17), 73.3 (C20), 69.2 (C16), 61.7 (C26), 57.5 (C24), 56.3 (C25).

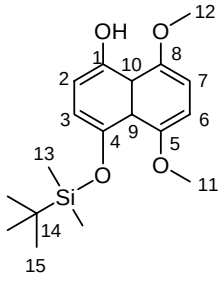


4.156b

(2*S*, 3*S*, 4*S*)-3,4-Bis(benzyloxy)-3,4-dihydro-2-methyl-6-phenyl-2H-pyran (4.156b). (5-86). A solution of *sec*-BuLi (3.1 mL, 1.3 M, 4.0 mmol) in cyclohexane was added dropwise to a stirred solution of 2-chloro-1,4-dimethoxybenzne (690 mg, 4.0 mmol) in THF (9 mL) at -95 ºC. The mixture was stirred for 15 min at -95 ºC, whereupon 2-triisopropylsilyloxyfuran (500 mg, 2.08 mmol) was added dropwise. The reaction was allowed to warm up rapidly to 0 ºC, whereupon saturated $NaHCO_3$ (10 mL) was added. The layers were separated, and the aqueous layer was extracted with Et_2O (3 x 15 mL). The combined organic layers were dried (Na_2SO_4), and concentrated under reduced pressure. The residue was dissolved in CH_3CN (20 mL) and extracted with hexanes (2 x 15 mL), the combined organic layers were dried to afford 704 mg (90%) of **4.156b** as a pale yellow solid. ^{1}H NMR (400 MHz) δ 6.99 (dd, *J* = 5.5, 2.3 Hz, 1 H), 6.83 (d, *J* = 5.5

Hz, 1 H), 6.53 (d, *J* = 8.9 Hz, 1 H), 6.49 (d, *J* = 8.9 Hz, 1 H), 5.64 (d, *J* = 2.3 Hz, 1 H), 3.74 (s, 3 H), 3.73 (s, 3 H), 1.26-1.12 (comp, 3 H), 1.12-1.00 (comp, 18 H); ^{13}C NMR (100 MHz) δ 148.7, 147.1, 144.6, 144.4, 139.3, 112.7, 112.4, 111.4, 75.0, 56.7, 56.2, 17.87, 17.86, 12.8; IR ($CHCl_3$) 2943, 1496, 1318, 1256, 1066 cm^{-1}.

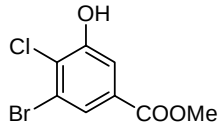
NMR assignments. ^{1}H NMR (400 MHz) δ 6.99 (dd, *J* = 5.5, 2.3 Hz, 1 H., C2-H), 6.83 (d, *J* = 5.5 Hz, 1 H, C3-H), 6.53 (d, *J* = 8.9 Hz, 1 H, C6-H or C7-H), 6.49 (d, *J* = 8.9 Hz, 1 H, C6-H or C7-H), 5.64 (d, *J* = 2.3 Hz, 1 H, C1-H), 3.74 (s, 3 H, C9-H or C10-H), 3.73 (s, 3 H, C9-H or C10-H), 1.26-1.12 (comp, 3 H, C11-H), 1.12-1.00 (comp, 18 H, C12-H); ^{13}C NMR (100 MHz) δ 148.7, 147.1, 144.6, 144.4, 139.3, 112.7, 112.4, 111.4, 75.0, 56.7, 56.2, 17.87, 17.86, 12.8.



4.157b

4-*tert*-Butyldimethylsilyloxy-5,8-dimethoxynaphthalen-1-ol (4.157b). (5-62). A solution of *sec*-BuLi (2.3 mL, 1.3 M, 3.0 mmol) in cyclohexane was added dropwise to a stirred solution of 2-chloro-1,4-dimethoxybenzne (518 mg, 3.0 mmol) in THF (7 mL) at –95 ºC. The mixture was stirred for 15 min at -95 ºC, whereupon 2-*tert*-butyldimethylsilylloxyfuran (200 mg, 1.0 mmol) was added dropwise. The reaction was allowed to warm up rapidly to 0 ºC, whereupon saturated $NaHCO_3$ (15 mL) was added.

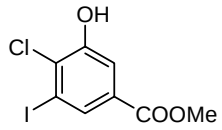
The layers were separated, and the aqueous layer was extracted with Et_2O (3 x 15 mL). The combined organic layers were dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by flash chromatography on basic alumina eluting with Et_2O/hexanes (1:20) to afford 342 mg (90%) of **4.157b** as a white solid. ^1H NMR (250 MHz) δ 9.42 (d, 1 H), 6.80 (d, *J* = 8.4 Hz, 1 H), 6.74 (d, *J* = 8.4 Hz, 1 H), 6.64 (d, *J* = 8.6 Hz, 1 H), 6.58 (d, *J* = 8.6 Hz, 1 H), 3.95 (s, 3 H), 3.81 (s, 3 H), 1.01 (comp, 9 H), 0.14 (s, 6 H); ^{13}C NMR (63 MHz) δ 151.8, 149.9, 148.6, 121.3, 118.4, 117.1, 111.1, 104.3, 104.2, 56.4, 56.6, 26.0, 25.6, 18.5, -4.5.



4.168

Methyl 3-bromo-4-chloro-5-hydroxybenzoate (4.168). (4-207, 4-218, 4-220). A cold solution of $NaNO_2$ (1.15 g, 16.7 mmol) in H_2O (5 mL) was added to a suspension of amine **4.165** (3.0 g, 13.9 mmol) in HBr (48% aqueous, 20 mL) at 0 °C. The mixture was stirred at 0°C for 40 min and it was then added slowly to a solution of CuBr (1.32 g, 9.17 mmol) in HBr (48% aquaous, 4 mL) at 100 °C. The mixture was hearted at 100 °C for 15 min and cooled to rt. The solid/liquid mixture was extracted with CH_2Cl_2 (2 x 50 mL) and ether (50 mL). The combined organic layers were washed with H_2O (50 mL) and $NaHCO_3$ (50 mL). The organic layers was dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by flash chromatography eluting with Et_2O/hexanes (1:10 then 1:7) to afford 3.05 g (79 %) of **4.166** as a white solid.

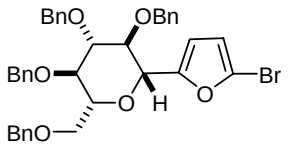
A solution of BBr_3 (4.38 g, 17.5 mmol) and **4.166** (2.87 g, 10.3 mmol) was stirred at rt overnight, whereupon MeOH was slowly added and the mixture was concentrated under reduced pressure to dryness. The residue was dissolved in MeOH (100 mL) containing conc. H_2SO_4 (10 d) and heated at reflux for 6 h. The mixture was cooled to rt and concentrated under reduced pressure. The residue was dissolved in EtOAc (50 mL) and washed with NaHCO3 (50 mL) and NaCl (50 mL). The organic layer was concentrated and purified by crystallization from hexanes/$CHCl_3$ to afford 2.45 g (91 %) of **4.168** as a pink solid: ^{1}H NMR (400 MHz, acetone-d_6) δ 7.73 (br s, 1 H), 7.60 (br s , 1 H), 3.87 (s, 3 H); ^{13}C NMR (100 MHz, acetone-d_6) δ 165.3, 155.3, 131.2, 126.9, 125.5, 123.8, 116.7, 52.8.



4.169

Methyl 3-iodo-4-chloro-5-hydroxybenzoate (4.169). (4-206, 4-211, 4-216). A cold solution of $NaNO_2$ (1.15 g, 16.7 mmol) in H_2O (20 mL) was added to a suspension of amine **4.165** (3.0 g, 13.9 mmol) in HCl (6 M, 60 mL) at 0 °C. The mixture was stirred at 0°C for 45 min, whereupon a solution of KI (3.46 g, 20.9 mmol) in H_2O (30 mL) was added slowly. The mixture was slowed to warm to rt and stirred for 20 min. The mixture was extracted with Et_2O (100 mL). The organic layers were washed with H_2O (50 mL) $NaHCO_3$ (50 mL), $Na_2S_2O_3$ (50 mL) and NaCl (50 mL). The organic layers was dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by flash chromatography eluting with Et_2O/hexanes (1:7) to afford 3.3 g (73 %) of **4.167** as a white solid.

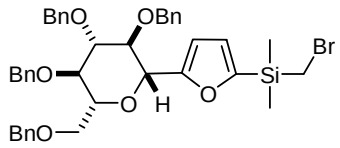
By applying similar method to make **4.168,** alcohol **4.169** was prepared from methyl ether **4.167** (168 mg, 0.515 mmol), BBr_3 (53 μL, 0.567 mmol) as a light brown solid (80%, 128 mg): ^{1}H NMR (400 MHz, acetone-d_6) δ 7.97 (d, J = 1.9 Hz, 1 H), 7.60 (d, J = 1.9 Hz, 1 H), 387 (s, 3 H); ^{13}C NMR (100 MHz, acetone-d_6) δ 165.2, 154.1, 132.2, 131.6, 130.7, 117.5, 99.5, 52.7.



4.180

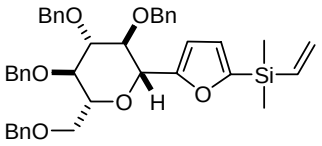
(2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5-Tris(benzyloxy)-2-(benzyloxymethyl)-6-(5-bromofuran-2-yl)-tetrahydro-2H-pyran (4.180). (5-282). A solution of Br_2 (96 μL, 1.87 mmol) in CCl_4 (1 mL) was added to glycsyl furan **4.104** (1.00 g, 1.70 mmol) in CCl_4 (20 mL). The mixture was stirred at rt for 5 min. The reaction was quenched by addition of saturated $NaHCO_3$ (40 mL). The layers were separated, and the organic layer was washed with $NaHCO_3$ (25 mL) and brine (25 mL). The organic layers was dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by flash chromatography eluting with Et_2O/hexanes (1:3) to afford 1.023 g (90 %) of **4.180** as a light yellow solid: ^{1}H NMR (400 MHz) δ 7.38-7.20 (comp, 16 H), 7.17-7.12 (comp, 2 H), 7.06-7.01 (comp, 2 H), 6.41 (d, J = 3.5 Hz, 1 H), 6.32 (d, J = 3.5 Hz, 1 H), 4.95 (d, J = 11.0 Hz, 1 H), 4.89 (d, J = 11.3 Hz, 1 H), 4.83 (d, J = 10.6 Hz, 1 H), 4.62-4.51 (comp, 4 H), 4.26 (d, J = 9.9 Hz, 1 H), 4.17 (d, J = 10.6 Hz, 1 H), 3.87 (appt, J = 9.2 Hz, 1 H), 3.78-3.65 (comp, 4 H), 3.59-3.52 (m, 1 H); ^{13}C NMR (100 MHz) δ 153.3, 138.5, 138.0, 137.9, 137.6, 128.4, 128.3, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 121.9, 112.7, 112.2, 86.5, 80.6, 79.3, 77.9, 75.5, 75.1, 74.8, 74.6, 73.4, 68.9; IR ($CHCl_3$) 3030, 2864, 1496,

1453, 1360, 1069 cm^{-1}; MS (CI) *m/z* 669.1824 [$C_{38}H_{38}O_6Br$ (M+1) requires 669.1852] (base), 671, 589.



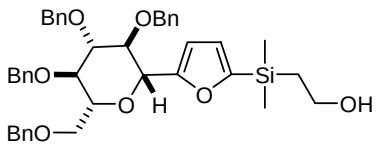
4.181

(5-(2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5-Tris(benzyloxy)-6-benzyloxymethyl-tetrahydro-2H-pyran-2-yl)furan-2-yl)(bromomethyl)dimethylsilane (4.181). (6-29). *n*-BuLi in hexanes (2.6 M, 1.60 mL, 4.16 mmol) was added to to **4.180** (2.428 g, 3.63 mmol) in THF (50 mL) at –78 °C. The mixture was stirred at –78 °C for 40 min whereupon bromomethylchlorodimethylsilane (570 μL, 4.18 mmol) was then added dropwise, the mixture was then allowed to warm to rt and stirred for 4 h at rt. The reaction was quenched by addition of saturated $NaHCO_3$ (50 mL). The layers were separated, and the aqueous layer was extracted with Et_2O (2 x 50 mL). The combined organic layers were dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by flash chromatography eluting with Et_2O/hexanes (1:3) to afford 2.606 g (97%) of **4.181** as a thick oil: ^1H NMR (400 MHz) δ 7.40-7.16 (comp, 20 H), 7.02-6.97 (comp, 2 H), 6.79 (d, *J* = 3.4 Hz, 1H), 6.51 (d, *J* = 3.4 Hz, 1 H), 4.97 (d, *J* = 10.9 Hz, 1 H), 4.95-4.84 (comp, 2 H), 4.67 (d, *J* = 12.3 Hz, 1 H), 4.63 (d, *J* = 10.9 Hz, 1 H), 4.60 (d, *J* = 12.3 Hz, 1 H), 4.54 (d, *J* = 10.9 Hz, 1 H), 4.41 (d, *J* = 9.6 Hz, 1 H), 4.04 (d, *J* = 10.3 Hz, 1 H), 3.97-3.92 (m, 1 H), 3.83-3.72 (comp, 4 H), 3.64-3.57 (m, 1 H), 2.61 (s, 2 H), 0.44 (s 3 H), 0.43 (s, 3 H); ^{13}C NMR (100 MHz) δ 156.4, 56.3, 138.6, 138.1, 138.0, 137.8, 128.4, 128.3, 128.2, 128.0, 127.9, 127.82, 127.76, 127.7, 127.6, 127.56, 127.46, 122.5, 110.2, 86.5, 81.6, 79.4, 78.0, 75.6, 75.1, 74.8, 74.7, 73.4, 68.9, 15.6, -4.21, -4.24; mass spectrum (CI) *m/z* 740.2152 [$C_{41}H_{45}O_6SiBr$ (M) requires 740.2169] 743 (base), 741, 725.



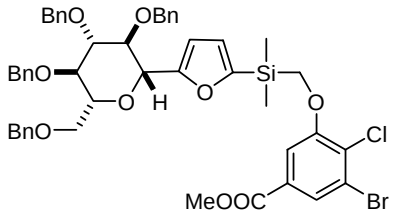
4.184

5-(2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5-Tris(benzyloxy-6-(benzyloxymethyl)-tetrahydro-2H-pyran-2-yl)furan-2-yl)dimethylvinylsilane (4.184). (5-289). *n*-BuLi in hexanes (2.45 M, 0.73 mL, 1.79 mmol) was added to to **4.180** (1.00 g, 1.49 mmol) in THF (20 mL) at –78 °C. The mixture was stirred at –78 °C for 60 min whereupon chlorodimethylvinylsilane (250 μL, 1.81 mmol) was then added dropwise, the mixture was then allowed to warm to rt and stirred overnight at rt. The reaction was quenched by addition of saturated $NaHCO_3$ (40 mL). The layers were separated, and the aqueous layer was extracted with Et_2O (2 x 30 mL). The combined organic layers were dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by flash chromatography eluting with Et_2O/hexanes (1:3) to afford 0.877 g (87%) of **4.184** as a thick oil: ^{1}H NMR (300 MHz) δ 7.36-7.12 (comp, 18 H), 7.00-6.94 (comp, 2 H), 6.67 (d, *J* = 3.1 Hz, 1 H), 6.46 (d, *J* = 3.1 Hz, 1 H), 6.22 (dd, *J* = 20.1, 14.7 Hz, 1 H), 6.01 (dd, *J* = 14.7, 3.8 Hz, 1 H), 5.78 (dd, *J* = 20.1, 3.8 Hz, 1 H), 4.94 (d, *J* = 10.9 Hz, 1 H), 4.88 (d, *J* = 10.9 Hz, 1 H), 4.86 (d, *J* = 10.6 Hz, 1 H), 4.63 (d, *J* = 12.0 Hz, 1 H), 4.60 (d, *J* = 10.6 Hz, 1 H), 4.55 (d, *J* = 12.0 Hz, 1 H), 4.37 (d, *J* = 9.5 Hz, 1 H), 4.01 (d, *J* = 10.6 Hz, 1 H), 3.96-3.90 (m, 1 H), 3.80-3.70 (comp, 4 H), 3.60-3.54 (m, 1 H), 0.33 (s, 6 H); mass spectrum (CI) *m/z* 675.3142 [$C_{42}H_{47}O_6Si$ (M+1) requires 675.3142], 567 (base).



4.185

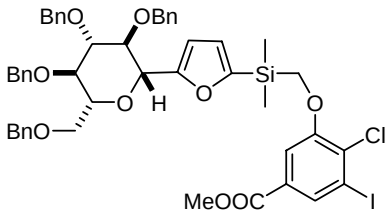
2-((5-(2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5-Tris(benzyloxy)-6-(benzyloxymethyl)-tetrahydro-2H-pyran-2-yl)furan-2-yl)dimethylsilyl)ethanol (4.185). (5-296). A solution of vinylsilane **4.184** (100 mg, 0.148 mmol) and 9-BBN (45 mg, 0.37 mmol) in THF (1 mL) was stirred overnight. A NaOH solution (3 N, 0.4 mL) was added at rt, then 30% H_2O_2 (0.2 mL) was added after 15 sec. The mixture was stirred for 30 min. The mixture was extracted with ether (2 x 7 mL) and then EtOAc (7 mL). The organic layers were combined, dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue was purified by chromatography eluting with Et_2O/hexanes (1:1) to yield 84 mg (82%) of **4.185** as a clear liquid: ^{1}H NMR (400 MHz) δ 7.40-7.20 (comp, 18 H), 7.04-7.00 (comp, 2 H), 6.71 (d, *J* = 3.2 Hz, 1 H), 6.51 (d, *J* = 3.2 Hz, 1 H)), 5.01-4.80 (comp, 3 H), 4. 66 (d, *J* = 12.3 Hz, 1 H), 4.65 (d, *J* = 10.6 Hz, 1 H), 4.61-4.53 (comp, 2 H), 4.42 (d, *J* = 10.0 Hz, 1 H), 4.06 (d, *J* = 10.6 Hz, 1 H), 4.00-3.92 (m, 1 H), 3.84-3.72 (comp, 4 H), 3.75 (t, *J* = 7.5 Hz, 2 H), 3.66-3.58 (m, 1 H), 1.19 (app t, *J* = 7.5 Hz, 2 H), 0.32 (s, 3 H), 0.31 (s, 3 H); ^{13}C NMR (100 MHz) δ 158.8, 155.8, 138.6, 138.1, 138.0, 137.9, 128.4, 128.3, 128.2, 128.0, 127.9, 127.8, 127.72, 127.68, 127.6, 127.5, 121.3, 1100, 86.5, 81.6, 79.3, 78.0, 75.6, 75.1, 74.7, 74.6, 73.4, 68.9, 59.5, 20.4, -3.0, -3.1; mass spectrum (CI) *m/z* 693.3241 [$C_{42}H_{49}O_7Si$ (M+1) requires 693.3246] (base), 585, 483.



4.182

Methyl 3-((5-(2*R*,3*R*,4*S*,5*R*,*6R*)-3,4,5-tris(benzyloxy)-6-(benzyloxymethyl)-tetrahydro-2H-pyran-2-yl)furan-2-yl)dimethylsilylmethoxy-5-bromo-4-

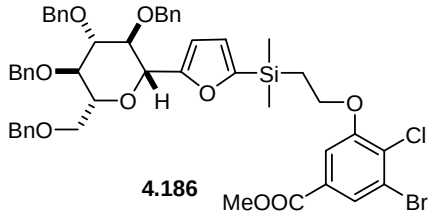
chlorobenzoate (4.182). (6-23). A mixture of tetrabutylammonium iodide (TBAI) (10.2 mg, 0.0276 mmol), Na_2CO_3 (12.3 mg, 0.115 mmol), bromomethylsilane **4.181** (17 mg, 0.023 mmol), and phenol **4.168** (6.7 mg, 0.025 mmol) in DMF (0.3 mL) was heated at 70 °C for 7 h. The mixture was alloed to cool to rt and was directly poured on top of a column and purified by flash chromatography eluting with Et_2O/hexanes (1:4 then 1:2) to give 20 mg (94%) of **4.182** as a colorless oil: ^{1}H NMR (400 MHz) δ 7.85 (dd, *J* = 1.7, 0.7 Hz, 1 H), 7.53 (d, *J* = 1.7 Hz, 1 H), 7.38-7.24 (comp, 13 H), 7.18-7.12 (comp, 5 H), 6.92-6.86 (comp, 2 H), 6.82 (d, *J* = 3.0 Hz, 1 H), 6.49 (d, *J* = 3.0 Hz, 1 H), 4.92 (d, *J* = 11.1 Hz, 1 H), 4.86 (d, *J* = 11.1 Hz, 1 H), 4.85 (d, *J* = 10.8 Hz, 1 H), 4.61 (d, *J* = 11.7 Hz, 1 H), 4.59 (d, *J* = 10.8 Hz, 1 H), 4.53 (d, *J* = 11.7 Hz, 1 H), 4.46 (d, *J* = 10.3 Hz, 1 H), 4.38 (d, *J* = 9.6 Hz, 1 H), 3.99 (d, *J* = 10.6 Hz, 1 H), 3.94-3.88 (m, 1 H), 3.89 (s, 3 H), 3.82 (s, 2 H), 3.77-3.72 (comp, 4 H), 3.60-3.54 (m, 1 H), 0.46 (s, 3 H), 0.45 (s, 3 H); ^{13}C NMR (100 MHz) δ 165.3, 157.6, 156.4, 156.0, 138.6 138.1, 138.0, 137.8, 129.6, 128.6, 128.4, 128.3, 128.2, 128.0, 127.83, 127.76, 127.7, 127.6, 127.5, 126.1, 123.5, 122.8, 111.4, 110.4, 86.5, 81.6, 79.4, 78.0, 75.6, 75.1, 74.7, 73.4, 68.9, 61.4, 52.5, -4.9.



4.183

Methyl 3-((5-(2*R*,3*R*,4*S*,5*R*,*6R*)-3,4,5-Tris(benzyloxy)-6-(benzyloxymethyl)-tetrahydro-2H-pyran-2-yl)furan-2-yl)dimethylsilylmethoxy-5-iodo-4-chlorobenzoate (4.183). (6-35). A mixture of tetrabutylammonium iodide (TBAI) (306 mg, 0.83 mmol), Na_2CO_3 (370 mg, 3.49 mmol), bromomethylsilane **4.181** (510 mg, 0.69 mmol), and

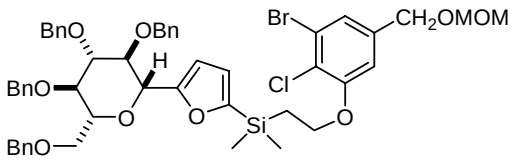
phenol **4.169** (240 mg, 0.77 mmol) in DMF (9 mL) was heated at 70 °C for 8 h. The mixture was alloed to cool to rt and H_2O (15 mL) was added, and the aqueous mixture was extracted with Et_2O (20 mL) and then EtOAc (2 x 15 mL). The combined organic layers were washed with H_2O (20 mL), 1 N NaOH (20 mL), brine (20 mL), dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by flash chromatography eluting with Et_2O/hexanes (1:3 then 1:2) to give 478 mg (70%) of **4.183** as a colorless oil: ^{1}H NMR (400 MHz) δ 8.07 (d, *J* = 1.6 Hz, 1 H), 7.55 (d, *J* = 1.6 Hz, 1 H), 7.38-7.24 (comp, 13 H), 7.20-7.12 (comp, 5 H), 6.92-6.87 (comp, 2 H), 6.83 (d, *J* = 3.1 Hz, 1 H), 6.50 (d, *J* = 3.1 Hz, 1 H), 4.92 (d, *J* = 10.9 Hz, 1 H), 4.87 (d, *J* = 10.9 Hz, 1 H), 4.86 (d, *J* = 10.6 Hz, 1 H), 4.61 (d, *J* = 12.2 Hz, 1 H), 4.59 (d, *J* = 10.6 Hz, 1 H), 4.55 (d, *J* = 12.2 Hz, 1 H), 4.46 (d, *J* = 10.4 Hz, 1 H), 4.39 (d, *J* = 9.9 Hz, 1 H), 3.99 (d, *J* = 10.4 Hz, 1 H), 3.95-3.90 (m, 1 H), 3.89 (s, 3 H), 3.85-3.78 (comp, 2 H), 3.77-3.72 (comp, 4 H), 3.62-3.55 (m, 1 H), 0.46 (s, 3 H), 0.45 (s, 3 H); ^{13}C NMR (100 MHz) δ 165.0, 156.6, 156.3, 156.0, 138.5 138.0, 137.9, 137.7, 132.33, 132.27, 130.0, 128.3, 128.2, 128.1, 127.9, 127.8, 127.7, 127.59, 127.56, 127.5, 122.7, 112.2, 110.3, 99.1, 86.4, 81.5, 79.3, 77.9, 75.5, 75.0, 74.6, 73.3, 68.8, 61.2, 52.3, -5.0.



4.186

Methyl 3-(2-((5-((2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5-tris(benzyloxy)-6-(benzyloxymethyl)-tetrahydro-2H-pyran-2-yl)furan-2-yl)dimethylsilylethoxy)-5-bromo-4-chlorobenzoate (4.186). (6-150). Alcohol **4.185** (25 mg, 0.036 mmol), phenol **4.168** (19.1 mg, 0.072 mmol) and PPh_3 (12.3 mg, 0.047 mmol) were dissolved in toluene (0.35

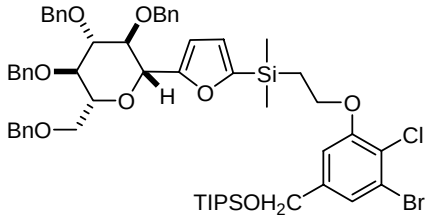
mL) and DIAD (7.5 μL, 0.038 mmol) was added dropwise at 0 °C. The solution was then heated at 80 °C for 2 h and then cooled. The mixture was purified without concentration by chromatography on silica gel eluting with Et_2O/hexanes (1:6 then 2:5) to afford 17 mg (50%) of aryl ether **4.186** as a clear oil. ^{1}H NMR (400 MHz) δ 7.87 (d, *J* = 1.6 Hz, 1 H), 7.41 (d, *J* = 1.6 Hz, 1 H), 7.38-7.22 (comp, 13 H), 7.22-7.12 (comp, 5 H), 6.96-6.88 (comp, 2 H), 6.72 (d, *J* = 3.1 Hz, 1 H), 6.47 (d, *J* = 3.1 Hz, 1 H), 4.92 (d, *J* = 11.1 Hz, 1 H), 4.87 (d, *J* = 11.1 Hz, 1 H), 4.85 (d, *J* = 10.6 Hz, 1 H), 4.61 (d, *J* = 12.0 Hz, 1 H), 4.60 (d, *J* = 10.6 Hz, 1 H), 4.54 (d, *J* = 12.0 Hz, 1 H), 4.48 (d, *J* = 10.3 Hz, 1 H), 4.37 (d, *J* = 9.9 Hz, 1 H), 4.22-4.02 (comp, 2 H), 3.99 (d, *J* = 10.3 Hz, 1 H), 3.92-3.82 (m, 1 H), 3.89 (s, 3 H), 3.80-3.68 (comp, 4 H), 3.60-3.72 (m, 1 H), 0.36 (s, 6 H).



4.188

(5-((2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5-Tris(benzyloxy)-6-(benzyloxymethyl)-tetrahydro-2H-pyran-2-yl)furan-2-yl)(2-(3-bromo-2-chloro-5-(methoxymethoxymethyl)-phenoxy)ethyl)dimethylsilane (4.188). (6-168). Alcohol **4.185** (23 mg, 0.033 mmol), phenol **4.187** (18.7 mg, 0.0662 mmol) and PPh_3 (11.3 mg, 0.043 mmol) were dissolved in benzene (0.4 mL) and DIAD (8.5 μL, 0.043 mmol) was added dropwise at rt. The solution was then heated at 75 °C for 3 h and then cooled. The mixture was purified without concentration by chromatography on silica gel eluting with Et_2O/hexanes (1:6 then 1:2 then 2:3) to afford 26 mg (82%) of aryl ether **4.188** as a clear oil. ^{1}H NMR (400 MHz) δ 7.40-7.16 (comp, 19 H), 7.00-6.94 (comp, 2 H), 6.77 (d, *J* = 1.7 Hz, 1 H), 6.74 (d, *J* = 3.3 Hz, 1 H), 6.50 (d, *J* = 3.3 Hz, 1 H), 4.96 (d, *J* = 11.1 Hz, 1 H), 4.90 (d, *J* =

11.1 Hz, 1 H), 4.89 (d, *J* = 10.6 Hz, 1 H), 4.69 (s, 2 H), 4.66-4.60 (comp, 2 H), 4.56 (d, *J* = 10.1 Hz, 1 H), 4.51 (d, *J* = 10.3 Hz, 1 H), 4.48 (s, 3 H), 4.40 (d, *J* = 9.6 Hz, 1 H), 4.20-4.12 (comp, 2 H), 4.02 (d, *J* = 10.6 Hz, 1 H), 3.92 (appt, *J* = 9.4 Hz, 1 H), 3.82-3.70 (comp, 4 H), 3.64-3.56 (m, 1 H), 3.41 (s, 3 H), 1.46 (t, *J* = 7.9 Hz, 1 H), 0.39 (s, 3 H); ^{13}C NMR (100 MHz) δ 158.3, 156.1, 155.4, 138.6, 138.3, 138.1, 138.0, 137.8, 128.4, 128.3, 128.2, 128.0, 127.9, 127.8, 127.74, 127.70, 127.6, 127.5, 124.0, 123.6, 122.5, 121.6, 110.7, 110.1, 95.7, 86.5, 81.7, 79.4, 78.0, 75.6, 75.1, 74.73, 74.68, 73.4, 68.9, 67.9, 66.6, 55.5, 16.1, -2.9.

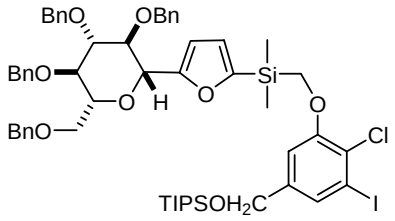


4.196

3,4,5-Tris(benzyloxy)-2-benzyloxymethyl-6-(5-{[2-(3-bromo-2-chloro-5-triisopropylsilanyloxymethylphenoxy)-ethyl]-dimethyl-silanyl}-furan-2-yl)-tetrahydropyran (4.196). (6-175, 6-177). A solution of ester **4.186** (132 mg, 0.140 mmol) in CH_2Cl_2 (10 mL) containg DIBAL-H (1 M, 32 μL, 0.32 mmol) was stirred at 0° for 15 min, whereupon saturated Rochelle's salt (15 mL) was added. The mixture was stirred overnight and the layers were separated. The aqueous layer was extracted with Et_2O (2 x 16 mL). The combined organic layers were dried (Na_2SO_4), concentrated under reduced pressure. The residue was purified by flashing chromatography, eluting with Et_2O/hexanes (2:3 to 3:2) to yield 98 mg of benzylic alcohol as clear liquid.

A solution of benzylic alcohol (98 mg, 0.107 mmol) obtained above, 2,6-lutidine (50 μL, 0.92 mmol), TIPSOTf (58 μL, 0.214 mmol) was stirred in CH_2Cl_2 (5 mL) at rt

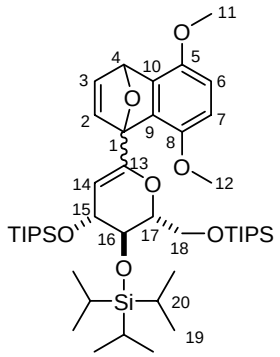
for 3 h. The reaction was quenched by addition of $NaHCO_3$ (10 mL) and the layers were separated. The aqueous layer was extracted with Et_2O (2 x 10 mL). The combined organic layers were dried (Na_2SO_4), concentrated under reduced pressure. The residue was purified by flashing chromatography, eluting with Et_2O/hexanes (1:5 to 1:4) to yield 116 mg (78% over two steps) of **4.196** as clear liquid: ^{1}H NMR (400 MHz) δ 7.36-7.24 (comp, 13 H), 7.20-7.12 (comp, 6 H), 6.96-6.90 (comp, 2 H), 6.84 (d, *J* = 1.0 Hz, 1 H), 6.69 (d, *J* = 3.4 Hz, 1 H), 6.46 (d, *J* = 3.4 Hz, 1 H), 4.92 (d, *J* = 11.1 Hz, 1 H), 4.87 (d, *J* = 11.1 Hz, 1 H), 4.85 (d, *J* = 10.6 Hz, 1 H), 4.71 (s, 2 H), 4.60 (d, *J* = 12.0 Hz, 1 H), 4.59 (d, *J* = 10.6 Hz, 1 H), 4.53 (d, *J* = 12.0 Hz, 1 H), 4.48 (d, *J* = 10.6 Hz, 1 H), 4.36 (d, *J* = 9.6 Hz, 1 H), 4.16-4.08 (comp, 2 H), 3.99 (d, *J* = 10.4 Hz, 1 H), 3.89 (t, *J* = 9.2 Hz, 1 H), 3.78-3.70 (comp, 4 H), 3.60-3.54 (m, 1 H), 1.43 (t, *J* = 7.9 Hz, 1 H), 1.20-1.12 (comp 21 H), 0.34 (s, 6 H); ^{13}C NMR (100 MHz) δ 158.3, 156.1, 155.4, 142.1, 138.6, 138.1, 138.0, 137.8, 128.4, 128.3, 128.2, 128.0, 127.9, 127.8, 127.7, 127.6, 123.3, 121.9, 121.6, 121.3, 110.1, 109.0, 86.5, 81.7, 79.4, 78.1, 75.6, 75.1, 74.8, 73.4, 71.8, 68.9, 66.5, 63.8, 59.1, 18.0, 16.1, 11.9, -3.0.



4.195

3,4,5-Tris(benzyloxy)-2-benzyloxymethyl-6-{5-[(2-chloro-3-iodo-5-triisopropylsilanyloxymethylphenoxymethyl)-dimethyl-silanyl]-furan-2-yl}-tetrahydropyran. (4.195). (6-48, 6-51). By applying the same method to make **4.196,** ether **4.195** was prepared from ester **4.183** (462 mg, 0.467 mmol) in 76% yield as a clear

liquid after purification by flash chromatography eluting with EtOAc/hexanes (1:10): ^{1}H NMR (400 MHz) δ 7.40-7.39 (m, 1 H), 7.36-7.224 (comp, 13 H), 7.18-7.14 (comp, 5 H), 6.94-6.88 (comp, 3 H), 6.82 (d, *J* = 3.2 Hz, 1 H), 6.49 (d, *J* = 3.2 Hz, 1 H), 4.92 (d, *J* = 11.1 Hz, 1 H), 4.87 (d, *J* = 11.1 Hz, 1 H), 4.85 (d, *J* = 10.8 Hz, 1 H), 4.68 (s, 2 H), 4.61 (d, *J* = 12.3 Hz, 1 H), 4.59 (d, *J* = 10.8 Hz, 1 H), 4.54 (d, *J* = 12.3 Hz, 1 H), 4.46 (d, *J* = 10.3 Hz, 1 H), 4.37 (d, *J* = 9.9 Hz, 1 H), 3.99 (d, *J* = 10.6 Hz, 1 H), 3.94-3.88 (m, 1 H), 3.80-3.70 (comp, 6 H), 3.60-3.54 (m, 1 H), 1.20-1.14 (comp, 21 H), 0.43 (s, 6 H); ^{13}C NMR (100 MHz) δ 156.6, 156.22, 156.18, 142.4, 138.5, 138.0, 137.9, 137.7, 128.24, 128.16, 128.07, 127.9, 127.74, 127.71, 127.60, 127.55, 127.5, 127.4, 125.2, 122.5, 110.2, 109.2, 99.1, 86.4, 81.5, 79.3, 77.9, 75.4, 74.9, 74.6, 73.3, 68.8, 63.6, 60.7, 17.9, 11.8, -5.0.

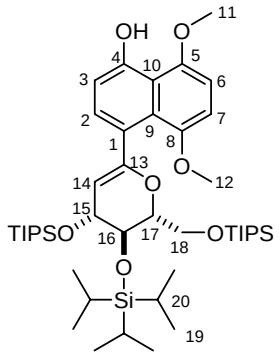


4.212

1,4-Dihydro-5,8-dimethoxy-1-(1,5-anhydro-3,4,6-*O*-(triisopropylsilyl)-2-deoxy-D-*arabino*-hexopyranosil)-4-naphthol (4.212). (3-280) Cycloadduct **4.212** was prepared in 89% yield (1:1 mixture of diastereomers) as pale yellow oil according to the general procedure described above. ^{1}H NMR (400 MHz) δ 7.24 (d, *J* = 5.5 Hz, 0.5 H, isomer A), 7.10 (dd, *J* = 5.5, 1.7 Hz, 0.5 H, isomer B), 7.06 (d, *J* = 5.5 Hz, 0.5 H, isomer B), 7.03 (dd, *J* = 5.5, 1.7 Hz, 0.5 H isomer A), 6.57 (d, *J* = 8.9 Hz, 0.5 H, isomer A), 6.56

(d, *J* = 8.9 Hz, 0.5 H, isomer B), 6.53 (d, *J* = 8.9 Hz, 0.5 H, isomer A), 6.52 (d, *J* = 8.9 Hz, 0.5 H, isomer B), 5.94 (d, *J* = 1.7 Hz, 0.5 H, isomer B), 5.92 (d, *J* = 1.7 Hz, 0.5 H, isomer A), 5.35 (dd, *J* = 5.1, 1.4 Hz, 0.5 H, isomer B), 5.17 (dd, *J* = 5.1, 1.4 Hz, 0.5 H, isomer A), 4.48-4.30 (comp, 3 H), 4.27-4.23 (m, 0.5 H, isomer B), 4.23-4.18 (m, 0.5 H, isomer A), 4.02-3.86 (m, 0.5 H, isomer A), 3.9-3.84 (m, 0.5 H, isomer B), 3.80 (s, 1.5 H, isomer A), 3.79 (s, 1.5 H, isomer B), 3.70 (s, 1.5 H, isomer A), 3.67 (s, 1.5 H, isomer B), 1.20-0.90 (comp, 63 H); mass spectrum (CI) *m/z* 817.5277 [$C_{45}H_{81}O_7Si_3$ (M+1) requires 817.5290], 774, 644 (base), 599, 386.

NMR assignments. ^{1}H NMR (400 MHz) δ 7.24 (d, *J* = 5.5 Hz, 0.5 H, isomer A, C2-H), 7.10 (dd, *J* = 5.5, 1.7 Hz, 0.5 H, isomer B, C3-H), 7.06 (d, *J* = 5.5 Hz, 0.5 H, isomer B, C2-H), 7.03 (dd, *J* = 5.5, 1.7 Hz, 0.5 H, isomer A, C3-H), 6.57 (d, *J* = 8.9 Hz, 0.5 H, isomer A, C6-H), 6.56 (d, *J* = 8.9 Hz, 0.5 H, isomer B, C6-H), 6.53 (d, *J* = 8.9 Hz, 0.5 H, isomer A, C7-H), 6.52 (d, *J* = 8.9 Hz, 0.5 H, isomer B, C7-H), 5.94 (d, *J* = 1.7 Hz, 0.5 H, isomer B, C4-H), 5.92 (d, *J* = 1.7 Hz, 0.5 H, isomer A, C4-H), 5.35 (dd, *J* = 5.1, 1.4 Hz, 0.5 H, isomer B, C14-H), 5.17 (dd, *J* = 5.1, 1.4 Hz, 0.5 H, isomer A, C14-H), 4.48-4.30 (comp, 3 H, C15-H & C18-H), 4.27-4.23 (m, 0.5 H, isomer B, C17-H), 4.23-4.18 (m, 0.5 H, isomer A, C17-H), 4.02-3.86 (m, 0.5 H, isomer A, C16-H), 3.9-3.84 (m, 0.5 H, isomer B, C16-H), 3.80 (s, 1.5 H, isomer A, C12-H), 3.79 (s, 1.5 H, isomer B, C12-H), 3.70 (s, 1.5 H, isomer A, C11-H), 3.67 (s, 1.5 H, isomer B, C11-H), 1.20-0.90 (comp, 63 H, C19-H & C20-H).

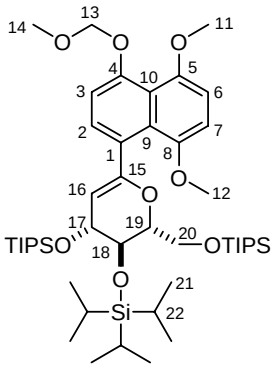


4.213

5,8-Dimethoxy-1-(1,5-anhydro-3,4,6-*O*-(triisopropylsilyl)-2-deoxy-D-***arabino*-**hex-1-enit-1-yl)-4-naphthol (4.213). (4-22).** To a solution of cycloadduct **4.212** (283 mg, 0.35 mmol) at –40 °C in CH_2Cl_2 (10 mL), was added $BF_3{\cdot}Et_2O$ (25 μL) dropwise. The solution quickly darkened, and the mixture was stirred at –40 °C for 15 min. The reaction was quenched by addition of saturated $NaHCO_3$ at –40 °C through syringe. The mixture was diluted with EtOAc (5 mL) and the layers were separated. The aqueous layer was extracted with EtOAc (3 x 15 mL). The combined organic layers were dried (Na_2SO_4), concentrated under reduced pressure. The residue was purified by flashing chromatography, eluting with EtOAc/hexanes (1:19) to yield 213 mg (75%) phenol **4.213** as clear liquid: ^{1}H NMR (400 MHz) δ 9.86 (s, 1 H), 7.32 (d, *J* = 8.2 Hz, 1 H), 6.85 (d, *J* = 8.2 Hz, 1 H), 6.74 (d, *J* = 8.6 Hz, 1 H), 6.71 (d, *J* = 8.6 Hz, 1 H), 4.81 (d, *J* = 4.4 Hz, 1 H), 4.42-4.24 (comp, 4 H), 4.03 (s, 3 H), 4.04-3.97 (m, 1 H), 3.83 (s, 3 H), 1.20-1.00 (comp, 63 H); ^{13}C NMR (100 MHz) δ 156.2, 155.2, 151.4, 150.1, 132.6, 126.3, 124.4, 116.1, 110.6, 105.2, 104.2, 96.5, 80.9, 68.8, 67.4, 61.9, 56.7, 56.6, 55.8, 18.3, 18.2, 18.1, 18.0, 12.6, 12.5, 12.0; mass spectrum (CI) *m/z* 817.5285 [$C_{45}H_{81}O_7Si_3$ (M+1) requires 817.5290] (base), 773, 643.

NMR assignments. ^{1}H NMR (400 MHz) δ 9.86 (s, 1 H, C4-OH), 7.32 (d, *J* = 8.2 Hz, 1 H, C2-H), 6.85 (d, *J* = 8.2 Hz, 1 H, C3-H), 6.74 (d, *J* = 8.6 Hz, 1 H, C7-H), 6.71 (d,

J = 8.6 Hz, 1 H, C6-H), 4.81 (d, *J* = 4.4 Hz, 1 H, C14-H), 4.42-4.24 (comp, 4 H, C15-H & C16-H & C17-H & C-18 H), 4.03 (s, 3 H, C12-H), 4.04-4.37 (m, 1 H, C18-H), 3.83 (s, 3 H, C11-H), 1.20-1.00 (comp, 63 H, sugar-OTIPS-H); ^{13}C NMR (100 MHz) δ 156.2 (C13), 155.2 (C4), 151.4 (C8), 150.1 (C5), 132.6 (C1), 126.3 (C2), 124.4 (C9), 116.1 (C10), 110.6 (C3), 105.2 (C7), 104.2 (C6), 96.5 (C14), 80.9 (C17), 68.8 (C16), 67.4 (C15), 61.9 (C18), 56.6 (C12), 55.8 (C11), 18.3 (C19), 18.2 (C19), 18.0 (C19), 12.6 (C20), 12.5 (C20), 12.0 (C20).

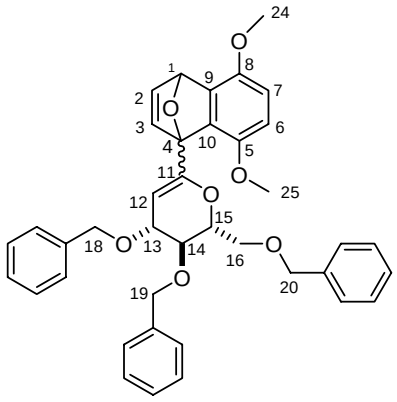


4.216

5,8-Dimethoxy-1-(1,5-anhydro-3,4,6-*O*-(triisopropylsilyl)-2-deoxy-D-*arabino*-hex-1-enit-1-yl)-4-methoxymethoxynaphthalene (4.216). (4-22) Phenol **4.213** (319 mg, 0.390 mmol) in THF (3 mL) was added to a suspension of NaH (24 mg, 60% suspension in mineral oil, 0.6 mmol) in THF (2 mL) at 0 °C. The mixture was stirred at rt for 30 min. The solution was cooled back down to 0 °C and MOMCl (48.3 mg, 0.60 mmol) was added. The mixture was stirred at rt for 1 h and *i*Pr_2NEt (26 mg, 0.2 mmol) was added. The mixture was stirred overnight and $NaHCO_3$ (10 mL) was added. The mixture was extracted with EtOAc (3 x 100 mL), and the combined organic layers were dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue was purified by

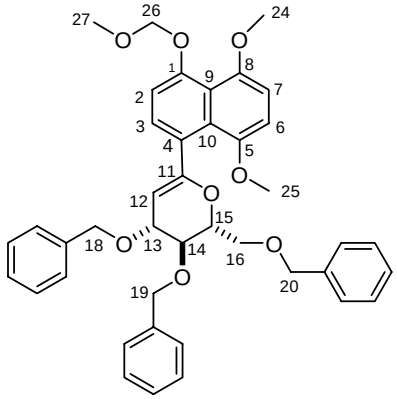
column chromatography, eluting with EtOAc/hexanes (100:3) to yield 281 mg (84%) of **4.216** as a clear liquid: ^{1}H NMR (400 MHz) δ 7.32 (d, *J* = 8.0 Hz, 1 H), 7.03 (d, *J* = 8.0 Hz, 1 H), 6.83 (d, *J* = 8.6 Hz, 1 H), 6.76 (d, *J* = 8.6 Hz, 1 H), 5.27 (s, 2 H), 4.81 (d, *J* = 5.1 Hz, 1 H), 4.60-4.20 (comp, 4 H), 4.04-3.92 (m, 1 H), 3.87 (s, 3 H), 3.81 (s, 3 H), 3.58 (s, 3 H), 1.18-0.98 comp, 63 H); ^{13}C NMR (100 MHz) δ 156.3, 154.2, 150.1, 150.7, 131.1, 127.9, 126.8, 120.3, 113.3, 108.5, 106.3, 96.8, 96.1, 80.9, 68.7, 67.1, 61.9, 57.9, 56.3, 55.9, 18.3, 18.2, 18.0, 17.2, 12.6, 12.0, 11.7; mass spectrum (CI) *m/z* 861.5527 [$C_{47}H_{85}O_8Si_3$ (M+1) requires 861.5552] (base), 687.

NMR assignments. ^{1}H NMR (400 MHz) δ 7.32 (d, *J* = 8.0 Hz, 1 H, C2-H), 7.03 (d, *J* = 8.0 Hz, 1 H, C3-H), 6.83 (d, *J* = 8.6 Hz, 1 H, C7-H), 6.76 (d, *J* = 8.6 Hz, 1 H, C6-H), 5.27 (s, 2 H, C13-H), 4.81 (d, *J* = 5.1 Hz, 1 H, C16-H), 4.60-4.20 (comp, 4 H, C17-H & C18-H & C19-H & C20-H), 4.04-3.92 (m, 1 H, C20-H), 3.87 (s, 3 H, C12-H), 3.81 (s, 3 H, C11-H), 3.58 (s, 3 H, C14-H); ^{13}C NMR (100 MHz) δ 156.3 (C15), 154.2 (C4), 150.1 (C8), 150.7 (C5), 131.1 (C1), 127.9 (C2), 126.8 (C9), 120.3 (C10), 113.3 (C3), 108.5 (C7), 106.3 (C6), 96.8 (C16), 96.1 (C13), 80.9 (C19), 68.7 (C18), 67.1 (C17), 61.9 (C20), 57.9 (C14), 56.3 (C12), 55.9 (C11), 18.3 (C21), 18.2 (C21), 18.0 (C21), 17.2 (C21), 12.6 (C22), 12.0 (C22), 11.7 (C22).



4.223

1-(4,5-Bis-benzyloxy-6-benzyloxymethyl-5,6-dihydro-4H-pyran-2-yl)-3,6-dimethoxy-11-oxa-tricyclo[6.2.1.02,7]undeca-2(7),3,5,9-tetraene. (4-223). Crude **4.223** was obtained in 95% yield as a thick oil from cycloaddition of glucosylfuran **4.222** (1.86 g, 3.86 mmol) and 2-chloro-1,4-dimethoxybenzne (2.0 g, 11.6 mmol) after purification by flash chromatography eluting with Et_2O/hexanes (from 1:2 to 2:3): ^1H NMR (400 MHz) δ 7.44-7.20 (comp, 15 H), 7.18-7.06 (comp, 2 H), 6.57 (app d, *J* = 8.4 Hz, 1 H), 6.53 (d, *J* = 8.6 Hz, 0.5 H), 6.53 (d, *J* = 8.6 Hz, 0.5 H), 6.96 (s, 1 H), 5.45 (d, *J* = 2.8 Hz, 0.5 H), 5.36 (d, *J* = 2.0 Hz, 0.5 H), 4.95 (d, *J* = 10.9 Hz, 0.5 H), 4.91 (d, *J* = 11.3 Hz, 0.5 H), 4.78 (d, *J* = 11.3 Hz, 0.5 H), 4.73 (d, *J* = 11.6 Hz, 0.5 H), 4.71 (d, *J* = 11.6 Hz, 0.5 H), 4.72-4.66 (m, 0.5 H), 4.63 (d, *J* = 11.6 Hz, 0.5 H), 4.65-4.58 (comp, 2 H), 4.54 (d, *J* = 12.0 Hz, 0.5 H), 4.48-4.42 (comp,1 H), 4.33-4.27 (m, 0.5 H), 4.26-4.20 (m , 0.5 H), 4.12 (dd, *J* = 9.8, 6.8 Hz, 0.5 H), 4.06 (dd, *J* = 8.9 Hz, 6.5 Hz, 0.5 H), 4.00-3.57 (comp, 2 H), 3.80 (s, 3 H), 3.63 (s, 3 H); IR (CH_2Cl_2) 2936, 1495, 1256 cm^{-1}; MS (CI) *m/z* 619.2705 [$C_{39}H_{39}O_7$ (M+1) requires 619.2696], 511, 309 (base).

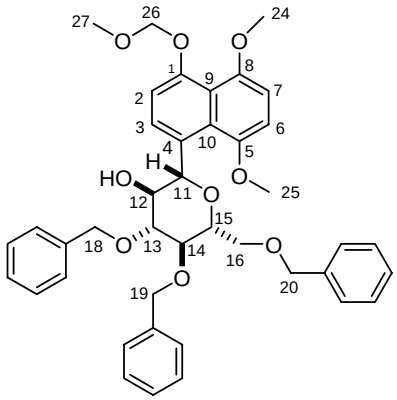


4.219

3,4-Bis(benzyloxy)-2-benzyloxymethyl-6-(5,8-dimethoxy-4-methoxymethoxy-naphthalen-1-yl)-3,4-dihydro-2H-pyran (4.219). (4-155, 4-157). A mixture of TIPS protected glucal **4.216** (893 mg, 1.04 mmol) and TBAF in THF (3.64 mL, 1 M, 3.64

mmol) in THF (20 mL) was stirred at rt for 3 h. The mixture was concentrated under reduced pressure and the residue was purified by flash chromatography eluting first with EtOAc then with EtOAc/MeOH (5:1) to afford 360 mg of crude **4.218** as a white solid.

NaH (181 mg, 4.54 mmol, 60% suspension in mineral oil) was added to a solution of triol **4.218** obtained above (360 mg, 0.918 mmol) and BnBr (0.50 mL, 4.14 mmol) in DMF (7 mL). The mixture was stirred at rt overnight. Saturated $NaHCO_3$ (40 mL) was added, and the mixture was extracted with EtOAc (3 x 30 mL). The combined organic layers were washed with H_2O (2 x 60 mL), dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by flash chromatography eluting with Et_2O/hexanes (1:1) to afford 527 mg (77% after two steps) of **4.219** as a thick oil: ^1H NMR (400 MHz) δ 7.50-7.26 (comp, 16 H), 7.10 (d, *J* = 7.9 Hz, 1 H), 6.89 (d, *J* = 8.5 Hz, 1 H), 6.84 (d, *J* = 8.5 Hz, 1 H), 5.31 (s, 2 H), 5.12-5.00 (br s, 1 H), 5.03 (d, *J* = 10.9 Hz, 1 H), 4.84-4.76 (comp, 2 H), 4.72 (d, *J* = 11.6 Hz, 1 H), 4.68-4.58 (m, 1 H), 4.62 (d, *J* = 12.0 Hz, 1 H), 4.54 (d, *J* = 12.0 Hz, 1 H), 4.40-4.30 (br d, 1 H), 4.15 (dd, *J* = 9.9, 6.9 Hz, 1 H), 3.94 (s, 3 H), 3.98-3.92 (comp, 2 H), 3.86 (s, 3 H), 3.64 (s, 3 H); ^{13}C NMR (75 MHz) δ 158.9, 154.6, 150.9, 150.5, 138.7, 138.4, 130.4, 128.3, 128.1, 127.9, 127.7, 127.6, 127.5, 127.4, 127.3, 127.0, 126.8, 120.2, 112.7, 108.2, 107.1, 96.6, 78.5, 78.0, 75.0, 74.0, 73.3, 70.1, 68.9, 57.6, 56.8, 56.3; IR (CH_2Cl_2) 2931, 1668, 1588, 1268, 1044 cm^{-1};mass spectrum (CI) *m/z* 663.2940 [$C_{41}H_{43}O_8$ (M+1) requires 663.2958] (base), 555.



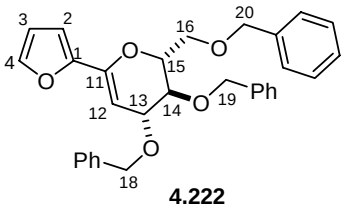
4.220

4,5-Bis(benzyloxy)-6-benzyloxymethyl-2-(5,8-dimethoxy-4-methoxymethoxy-naphthalen-1-yl)tetrahydropyran-3-ol (4.220). (4-109). A solution of glucal **4.219** (54 mg, 0.0816 mmol) and BH_3·DMS (25 μL, 10 M, 0.25 mmol) was stirred at rt for 20 h. A solution of NaOH (90 μL, 5 M, 0.45 mmol) and then H_2O_2 (90 μL, 30%) were added and the mixture was stirred at rt for 20 h. Saturated $NaHCO_3$ (10 mL) was added and the mixture was extracted with EtOAc (3 x 10 mL). The combined organic layers were dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by flash chromatography eluting with Et_2O/Hexanes (1:1) to afford 31 mg (56%) of **4.220** as a white solid: ^{1}H NMR (400 MHz) δ 7.75 (d, *J* = 8.2 Hz, 1 H), 7.41 (d, *J* = 6.8 Hz, 2 H), 7.36-7.22 (comp, 13 H), 7.18 (d, *J* = 8.5 Hz, 1 H), 6.83 (d, *J* = 8.5 Hz, 1 H), 6.80 (d, *J* = 8.5 Hz, 1 H), 5.81 (d, *J* = 8.9 Hz, 1 H), 5.24 (s, 2 H), 5.03 (d, *J* = 11.3 Hz, 1 H), 4.93 (d, *J* = 11.3 Hz, 1 H), 4.92 (d, *J* = 10.6 Hz, 1 H), 4.68-4.60 (comp, 2 H), 4.54 (d, *J* = 12.0 Hz, 1 H), 3.91 (s, 3 H), 3.89 (s, 3 H), 3.85-3.68 (comp, 6 H), 3.59 (s, 3 H), 2.65 (d, *J* = 5.1 Hz, 1 H); ^{13}C NMR (75 MHz) δ 153.8, 151.7, 150.5, 138.9, 138.3, 138.2, 129.2, 128.4, 128.3, 128.2, 128.0, 127.7, 127.6, 127.5, 126.5, 120.6, 114.4, 107.3, 107.2, 96.9, 87.7, 79.7, 78.4, 77.5, 77.2, 75.1, 73.4, 69.4, 57.6, 56.4, 55.9; IR (CH_2Cl_2) 3352, 2918, 1618,

1280, 1099, 1047 cm^{-1}; MS (CI) *m/z* 680.2993 [$C_{41}H_{44}O_9$ (M) requires 680.2985], 681 (base), 555.

NMR assignments. ^1H NMR (400 MHz) δ 7.75 (d, *J* = 8.2 Hz, 1 H, C3-H), 7.41 (d, *J* = 6.8 Hz, 2 H, benzyl aromatics), 7.36-7.22 (comp, 13 H, benzyl aromatics), 7.18 (d, *J* = 8.5 Hz, 1 H, C2-H), 6.83 (d, *J* = 8.5 Hz, 1 H, C7-H), 6.80 (d, *J* = 8.5 Hz, 1 H, C6-H), 5.81 (d, *J* = 8.9 Hz, 1 H, C4-H), 5.24 (s, 2 H, C26-H), 5.03 (d, *J* = 11.3 Hz, 1 H, C18-H), 4.93 (d, *J* = 11.3 Hz, 1 H, C18-H), 4.92 (d, *J* = 10.6 Hz, 1 H, C19-H), 4.68-4.60 (comp, 2 H, C19-H & C20-H), 4.54 (d, *J* = 12.0 Hz, 1 H, C20-H), 3.91 (s, 3 H), 3.89 (s, 3 H), 3.85-3.68 (comp, 6 H), 3.59 (s, 3 H), 2.65 (d, *J* = 5.1 Hz, 1 H, OH); ^{13}C NMR (75 MHz) δ 153.8, 151.7, 150.5, 138.9, 138.3, 138.2, 129.2, 128.4, 128.3, 128.2, 128.0, 127.7, 127.6, 127.5, 126.5, 120.6, 114.4, 107.3, 107.2, 96.9, 87.7, 79.7, 78.4, 77.5, 77.2, 75.1, 73.4, 69.4, 57.6, 56.4, 55.9.

General procedure for Converting 2-deoxyglucolactone to 1-furylglycal. To a solution of 2-deoxyglucolactone (0.175 mmol) in THF (1 mL) at –78 °C, was added 2-lithiofuran (640 μL, 0.296 M, 0.19 mmol), which was generated by adding *t*-BuLi (540 μL, 1.56 M, 0.84 mmol) to furan (56 μL, 77 mmol) in THF (2 mL) at –78 °C followed by stirring at –5 °C for 45 min. The mixture was stirred at –78 °C for 4.5 h, whereupon pyridine (43 μL, 0.53 mmol), DMAP (24 mg, 0.20 mmol) in THF (0.3 mL) and TFAA (58 μL, 0.44 mmol) was added sequentially. The cold bath was then packed with dry ice, stirred and warmed up overnight. Saturated $NaHCO_3$ (5 mL) was added and the mixture was extracted with EtOAc (3 x 5 mL). The combined organic layers were dried (Na_2SO_4), filtered and concentrated, and the residue was purified by flash chromatography, eluting with Et_2O/hexanes.



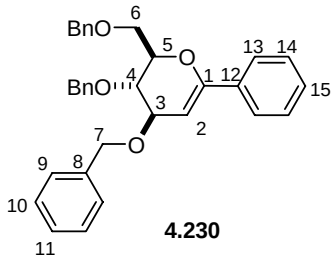
4.222

3,4-Bis(benzyloxy)-2-benzyloxymethyl-6-furan-2-yl-3,4-dihydro-2H-pyran (4.222). Method A **(4-163, 4-167)**: A mixture of TIPS protected glucal **4.211** (2.754 g, 4.05 mmol) and TBAF in THF (13.7 mL, 1 M, 13.7 mmol) in THF (45 mL) was stirred at rt for 4 h. The mixture was concentrated under reduced pressure, and the residue was purified by flash chromatography eluting with EtOAc to afford 920 mg of crude **4.221** as yellow powder.

NaH (920 mg, 23.0 mmol, 60% suspension in mineral oil) was added to a solution of triol **4.221** obtained above (920 mg) and BnBr (2.32 mL, 19.4 mmol) in DMF (25 mL). The mixture was stirred at rt overnight. Saturated $NaHCO_3$ (60 mL) was added, and the mixture was extracted with EtOAc (3 x 60 mL). The combined organic layers were washed with NaCl (3 x 80 mL), dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by flash chromatography eluting with Et_2O/hexanes (1:6) to afford 1.865 (96% after two steps) of **4.222** as a white solid.

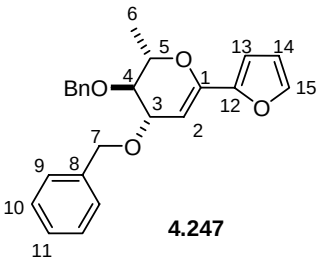
Method B (4-267-2): Glucal **4.222** was prepared in 75% yield from 2-deoxyglucolactone **4.224** (75 mg, 0.175 mmol) as thick oil according to the general procedure: ^{1}H NMR (400 MHz) δ 7.42-7.25 (comp, 16 H), 6.55 (d, J = 3.4 Hz, 1 H), 6.42 (dd, J = 3.4, 1.7 Hz, 1 H), 5.53 (d, J = 3.4 Hz, 1 H), 4.87 (d, J = 11.6 Hz, 1 H), 4.72 (d, J = 11.6 Hz, 1 H), 4.71 (d, J = 11.3 Hz, 1 H), 4.65-4.58 (comp, 3 H), 4.37 (dd, J = 5.8, 3.1

Hz, 1 H), 4.26 (ddd, *J* = 7.8, 4.8, 3.1 Hz, 1 H), 3.98 (dd, *J* = 8.2, 5.8 Hz, 1 H), 3.92 (dd, *J* = 10.9, 4.9 Hz, 1 H), 3.86 (dd, *J* = 10.9, 2.9 Hz, 1 H); ^{13}C NMR (100 MHz) δ 148.9, 145.3, 142.5, 138.3, 138.2, 138.1, 128.4, 128.3, 127.9, 127.7,2, 127.70, 127.62, 127.59, 127.54, 111.1, 107.6, 94.8, 75.6, 7.2, 73.5, 73.4, 70.2, 68.3; IR (CH_2Cl_2) 3029, 2864, 1668, 1565, 1453, 1094 cm^{-1}; MS (CI) *m/z* 483.2165 [$C_{31}H_{31}O_5$ (M+1) requires 483.2171], 375 (base), 253.



4.230

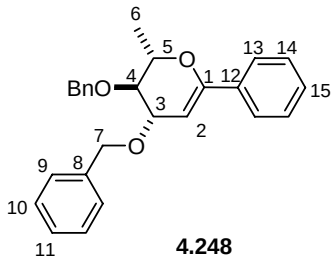
(2*R*, 3*S*, 4*R*)-3,4-Bis(benzyloxy)-2-((benzyloxy)methyl)-3,4-dihydro-6-phenyl-2H-pyran (4.230). (5-58). Glucal **4.230** was obtained as a clear oil in 60% yield from **4.224** (75 mg, 0.175 mmol) and phenyllithium (0.24 mmol) after purification by chromatography eluting with Et_2O/hexanes (1:7): ^{1}H NMR (400 MHz) δ 7.68-7.60 (comp, 2 H), 7.44-7.24 (comp, 18 H), 5.46 (d, *J* = 3.1 Hz, 1 H), 4.89 (d, *J* = 11.3 Hz, 1 H), 4.80-4.60 (comp, 5 H), 4.41 (dd, *J* = 5.8, 3.1 Hz, 1 H), 4.34-4.24 (m, 1 H), 4.06-3.88 (comp, 3 H); ^{13}C NMR (100 MHz) δ 152.7, 138.5, 138.2,134.5, 128.7, 128.4, 128.38, 128.3, 128.1, 127.9, 127.7, 127.6, 127.5, 125.2, 96.0, 76.6, 74.3, 73.5, 73.4, 70.4, 68.6; IR ($CHCl_3$) 2863, 1652, 1495, 1452, 1102, 695 cm^{-1}.



4.247

(2*S*, 3*S*, 4*S*)-3,4-Bis(benzyloxy)-6-(furan-2-yl)-3,4-dihydro-2-methyl-2H-pyran (4.247). (4-300). 6-Deoxyglucal **4.247** was obtained as a pale yellow wax in 92% yield from **4.246** (31 mg, 0.095 mmol) and 2-lithiofuran (0.10 mmol) after purification by flash chromatography eluting with Et_2O/hexanes (1:6): ^{1}H NMR (400 MHz) δ 7.43-7.27 (comp, 11 H), 6.50 (d, *J* = 3.4 Hz, 1 H), 6.40 (dd, *J* = 3.1, 1.7 Hz, 1 H), 5.51 (d, *J* = 3.1 Hz, 1 H), 4.92 (d, *J* = 11.3 Hz, 1 H), 4.76 (d, *J* = 11.3 Hz, 1 H), 4.74 (d, *J* = 11.3 Hz, 1 H), 4.63 (d, *J* = 11.6 Hz, 1 H), 4.39 (dd, *J* = 6.5, 3.0 Hz, 1 H), 4.12 (dq, *J* = 8.9, 6.2 Hz, 1 H), 3.59 (dd, *J* = 8.9, 6.5 Hz, 1 H), 1.48 (d, *J* = 6.5 Hz, 3 H); ^{13}C NMR (100 MHz) δ 150.0, 145.6, 142.5, 138.4, 138.2, 128.4, 128.0, 127.8, 127.7, 127.6, 111.1, 107.4, 95.1, 79.4, 76.7, 74.4, 73.9, 70.3, 17.5; IR ($CDCl_3$) 2867, 1667, 1491, 1110 cm-1; MS (CI) *m/z* 377.1741 [$C_{24}H_{25}O_4$ (M+1) requires 377.1753], 269 (base).

NMR assignments. **1**H NMR (400 MHz) δ 7.43-7.27 (comp, 11 H, aromatic H), 6.50 (d, *J* = 3.4 Hz, 1 H), 6.40 (dd, *J* = 3.1, 1.7 Hz, 1 H), 5.51 (d, *J* = 3.1 Hz, 1 H, C2-H), 4.92 (d, *J* = 11.3 Hz, 1 H, C7-H), 4.76 (d, *J* = 11.3 Hz, 1 H, C7-H), 4.74 (d, *J* = 11.3 Hz, 1 H, C7-H), 4.63 (d, *J* = 11.6 Hz, 1 H, C7-H), 4.39 (dd, *J* = 6.5, 3.0 Hz, 1 H, C3-H), 4.12 (dq, *J* = 8.9, 6.2 Hz, 1 H, C5-H), 3.59 (dd, *J* = 8.9, 6.5 Hz, 1 H, C4-H), 1.48 (d, *J* = 6.5 Hz, 3 H, C6-H); ^{13}C NMR (100 MHz) δ 150.0 (C1), 145.6, 142.5, 138.4, 138.2, 128.4, 128.0, 127.8, 127.7, 127.6, 111.1, 107.4, 95.1 (C2), 79.4 (C4), 76.7 (C3), 74.4, 73.9, 70.3 (C7), 17.5 (C6);

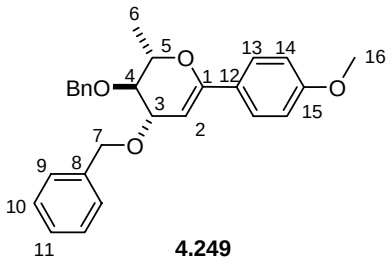


4.248

(2*S*, 3*S*, 4*S*)-3,4-Bis(benzyloxy)-3,4-dihydro-2-methyl-6-phenyl-2H-pyran (4.248). (5-45). 6-Deoxyglucal **4.248** was obtained as a white wax in 91% yield from **4.246** (51 mg, 0.174 mmol) and phenyllithium (0.225 mmol) after purification by flash chromatography eluting with Et_2O/hexanes (1:7): ^{1}H NMR (400 MHz) δ 7.63-7.57 (comp, 2 H), 7.44-7.28 (comp, 13 H), 5.44 (d, *J* = 2.9 Hz, 1 H), 4.94 (d, *J* = 11.6 Hz, 1 H), 4.76 (d, *J* = 11.3 Hz, 1 H), 4.75 (d, *J* = 11.3 Hz, 1 H), 4.68 (d, *J* = 11.6 Hz, 1 H), 4.42 (dd, *J* = 6.5, 2.9 Hz, 1 H), 4.15 (dq, *J* = 8.9, 6.3 Hz, 1 H), 3.61 (dd, *J* = 8.9, 6.5 Hz, 1 H), 1.52 (d, *J* = 6.3 Hz, 3 H); ^{13}C NMR (100 MHz) δ 152.9, 138.5, 138.3, 134.5, 128.6, 128.41, 128.39, 128.1, 128.0, 127.7, 127.6, 125.1, 96.2, 79.5, 77.5, 74.4, 73.9, 70.5, 17.6; IR ($CHCl_3$) 2870, 1650, 1495, 1451, 1112, 912 cm^{-1}; MS (CI) *m/z* 387.1964 [$C_{26}H_{27}O_3$ (M+1) requires 387.1960], 297, 279 (base).

NMR assignments. ^{1}H NMR (400 MHz) δ 7.63-7.57 (comp, 2 H, aromatic H), 7.44-7.28 (comp, 13 H, aromatic H), 5.44 (d, *J* = 2.9 Hz, 1 H, C2-H), 4.94 (d, *J* = 11.6 Hz, 1 H, C7-H), 4.76 (d, *J* = 11.3 Hz, 1 H, C7-H), 4.75 (d, *J* = 11.3 Hz, 1 H, C7-H), 4.68 (d, *J* = 11.6 Hz, 1 H, C7-H), 4.42 (dd, *J* = 6.5, 2.9 Hz, 1 H, C3-H), 4.15 (dq, *J* = 8.9, 6.3 Hz, 1 H, C5-H), 3.61 (dd, *J* = 8.9, 6.5 Hz, 1 H, C4-H), 1.52 (d, *J* = 6.3 Hz, 3 H, C6-H); ^{13}C NMR (100 MHz) δ 152.9 (C1), 138.5, 138.3, 134.5, 128.6, 128.41, 128.39, 128.1,

128.0, 127.7, 127.6, 125.1, 96.2 (C2), 79.5 (C4), 77.5 (C3), 74.4, 73.9, 70.5 (C7), 17.6 (C6).

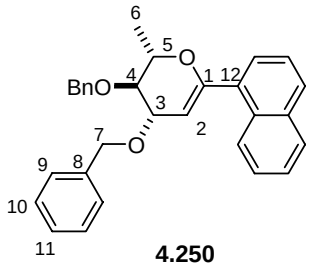


4.249

(2*S*, 3*S*, 4*S*)-3,4-Bis(benzyloxy)-3,4-dihydro-6-(4-methoxyphenyl)-2-methyl-2H-pyran (4.249). (5-68). 6-Deoxyglucal **4.249** was obtained as a pale yellow wax in 80% yield from **4.246** (57 mg, 0.174 mmol) and 4-methoxyphenyllithium (0.21 mmol) after purification by flash chromatography eluting with Et_2O/hexanes (1:7): ^{1}H NMR (400 MHz) δ 7.54 (d, *J* = 8.9 Hz, 2 H), 7.44-7.26 (comp, 10 H), 6.88 (d, *J* = 8.9 Hz, 2 H), 5.32 (d, *J* = 2.7 Hz, 1 H), 4.94 (d, *J* = 11.6 Hz, 1 H), 4.77 (d, *J* = 11.6 Hz, 1 H), 4.75 (d, *J* = 11.6 Hz, 1 H), 4.68 (d, *J* = 11.6 Hz, 1 H), 4.42 (dd, *J* = 6.5, 2.7 Hz, 1 H), 4.13 (dq, *J* = 8.9, 6.5 Hz, 1 H), 3.82 (s, 3 H), 3.60 (dd, *J* = 8.9, 6.5 Hz, 1 H), 1.51 (d, *J* = 6.5 Hz, 3 H); ^{13}C NMR (100 MHz) δ 160.0, 152.7, 138.6, 138.3, 128.4, 0, 128.38, 128.0, 127.7, 127.7, 127.6, 127.2, 126.5, 113.4, 94.6, 79.6, 77.6, 74.2, 73.9, 70.4, 55.2, 17.6; IR ($CHCl_3$) 2894, 1650, 1511, 1545, 1250, 1109 cm-1; MS (CI) *m/z* 417.2059 [$C_{27}H_{29}O_4$ (M+1) requires 417.2066], 309, 107 (base).

NMR assignments. ^{1}H NMR (400 MHz) δ 7.54 (d, *J* = 8.9 Hz, 2 H, C13-H), 7.44-7.26 (comp, 10 H, aromatic H), 6.88 (d, *J* = 8.9 Hz, 2 H, C14-H), 5.32 (d, *J* = 2.7

Hz, 1 H, C2-H), 4.94 (d, *J* = 11.6 Hz, 1 H, C7-H), 4.77 (d, *J* = 11.6 Hz, 1 H, C7-H), 4.75 (d, *J* = 11.6 Hz, 1 H, C7-H), 4.68 (d, *J* = 11.6 Hz, 1 H, C7-H), 4.42 (dd, *J* = 6.5, 2.7 Hz, 1 H, C3-H), 4.13 (dq, *J* = 8.9, 6.5 Hz, 1 H, C5-H), 3.82 (s, 3 H, C16-H), 3.60 (dd, *J* = 8.9, 6.5 Hz, 1 H, C4-H), 1.51 (d, *J* = 6.5 Hz, 3 H, C6-H); ^{13}C NMR (100 MHz) δ 160.0 (C1), 152.7 (C15), 138.6, 138.3, 128.4, 128.38, 128.0, 127.7, 127.7, 127.6, 127.2, 126.5, 113.4, 94.6, 79.6 (C4), 77.6 (C3), 74.2, 73.9, 70.4 (C7), 55.2 (C16), 17.6 (C6).

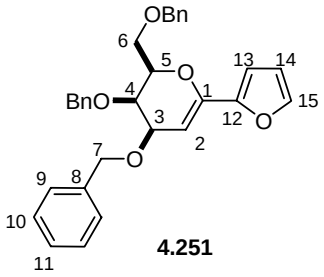


4.250

(2*S*, 3*S*, 4*S*)-3,4-Bis(benzyloxy)-3,4-dihydro-2-methyl-6-(naphthalen-1-yl)-2H-pyran (4.250). (5-69): 6-Deoxyglucal **4.250** was obtained as a pale yellow wax in 90% yield from **4.246** (57 mg, 0.174 mmol) and 1-naphthyllithium (0.121 mmol) after purification by chromatography eluting with Et_2O/hexanes (0:100 to 1:20 to 1:10): ^{1}H NMR (400 MHz) δ 8.18 (d, *J* = 1.7 Hz, 1 H), 7.88-7.80 (comp, 2 H), 7.60-7.28 (comp, 14 H), 5.25 (d, *J* = 2.7 Hz, 1 H), 5.00 (d, *J* = 11.3 Hz, 1 H), 4.83 (d, *J* = 11.3 Hz, 1 H), 4.77 (d, *J* = 11.6 Hz, 1 H), 4.68 (d, *J* = 11.6 Hz, 1 H), 4.49 (dd, *J* = 6.5, 2.7 Hz, 1 H), 4.34 (dq, *J* = 8.9, 6.5 Hz, 1 H), 3.76 (dd, *J* = 9.2, 6.5 Hz, 1 H), 1.55 (d, *J* = 6.5 Hz, 3 H); ^{13}C NMR (100 MHz) δ 154.4, 138.45, 138.40, 133.7, 131.2, 129.3, 128.48, 128.46, 128.3, 128.0, 127.9, 127.8, 127.7, 126.8, 126.3, 125.9, 125.7, 125.1, 101.1, 79.7, 77.5, 74.8, 74.0, 70.7,

17.8; IR ($CHCl_3$) 2868, 1660, 1453, 1100 cm-1; MS (CI) *m/z* 437.2119 [$C_{30}H_{29}O_3$ (M+1) requires 437.2114], 329 (base), 172.

NMR assignments. ^{1}H NMR (400 MHz) δ 8.18 (d, *J* = 1.7 Hz, 1 H, aromatic H), 7.88-7.80 (comp, 2 H, aromatic H), 7.60-7.28 (comp, 14 H, aromatic H), 5.25 (d, *J* = 2.7 Hz, 1 H, C2-H), 5.00 (d, *J* = 11.3 Hz, 1 H, C7-H), 4.83 (d, *J* = 11.3 Hz, 1 H, C7-H), 4.77 (d, *J* = 11.6 Hz, 1 H, C7-H), 4.68 (d, *J* = 11.6 Hz, 1 H, C7-H), 4.49 (dd, *J* = 6.5, 2.7 Hz, 1 H, C3-H), 4.34 (dq, *J* = 8.9, 6.5 Hz, 1 H, C5-H), 3.76 (dd, *J* = 9.2, 6.5 Hz, 1 H, C4-H), 1.55 (d, *J* = 6.5 Hz, 3 H, C6-H); ^{13}C NMR (100 MHz) δ 154.4 (C1), 138.45, 138.40, 133.7, 131.2, 129.3, 128.48, 128.46, 128.3, 128.0, 127.9, 127.8, 127.7, 126.8, 126.3, 125.9, 125.7, 125.1, 101.1 (C2), 79.7 (C4), 77.5 (C3), 74.8, 74.0, 70.7 (C7), 17.8 (C6).

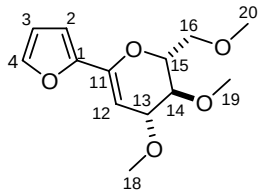


4.251

(2*R*, 3*R*, 4*R*)-3,4-Bis(benzyloxy)-2-(benzyloxy)methyl)-6-(furan-2-yl)-3,4-dihydro-2H-pyran (4.252). (4-301). Galactal **4.251** was obtained as a clear oil in 31% yield from **4.243** (75 mg, 0.175 mmol) and 2-lithiofuran (0.19 mmol) after purification by chromatography eluting with Et_2O/hexanes (1:6): ^{1}H NMR (400 MHz) δ 7.40-7.25 (comp, 16 H), 6.51 (d, *J* = 3.4 Hz, 1 H), 6.38 (dd, *J* = 3.4, 1.7 Hz, 1 H), 5.49 (d, *J* = 3.4 Hz, 1 H), 4.89 (d, *J* = 12.0 Hz, 1 H), 4.73 (d, *J* = 12.0 Hz, 1 H), 4.68 (d, *J* = 12.0 Hz, 1 H), 4.67 (d, *J* = 12.3 Hz, 1 H), 4.52 (d, *J* = 1.20 Hz, 1 H), 4.47 (d, *J* = 12.0 Hz, 1 H), 4.40-4.34 (m, 1 H), 4.34-4.30 (m, 1 H), 4.05-4.01 (m, 1 H), 3.88 (dd, *J* = 10.3, 6.8 Hz, 1 H), 3.81 (dd, *J* = 10.3, 5.1 Hz, 1 H); ^{13}C NMR (100 MHz) δ 149.2, 144.9, 142.6, 138.8,

1386, 138.4, 128.6, 128.5, 128.4, 128.0, 127.92, 127.89, 127.8, 127.7, 111.4, 107.6, 95.2, 76.5, 73.6, 73.4, 71.5, 71.1, 71.0, 68.4; IR ($CDCl_3$) 2865, 1665, 1493, 1094 cm-1; MS (CI) *m/z* 483.2164 [$C_{31}H_{31}O_5$ (M+1) requires 483.2171], 375 (base), 107.

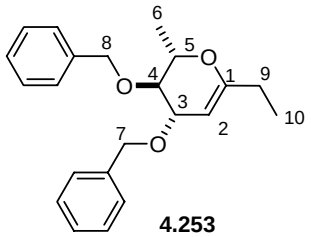
NMR assignments. ^{1}H NMR (400 MHz) δ 7.40-7.25 (comp, 16 H, aromatic H), 6.51 (d, *J* = 3.4 Hz, 1 H), 6.38 (dd, *J* = 3.4, 1.7 Hz, 1 H), 5.49 (d, *J* = 3.4 Hz, 1 H, C2-H), 4.89 (d, *J* = 12.0 Hz, 1 H, C7-H), 4.73 (d, *J* = 12.0 Hz, 1 H, C7-H), 4.68 (d, *J* = 12.0 Hz, 1 H, C7-H), 4.67 (d, *J* = 12.3 Hz, 1 H, C7-H), 4.52 (d, *J* = 1.20 Hz, 1 H, C7-H), 4.47 (d, *J* = 12.0 Hz, 1 H, C7-H), 4.40-4.34 (m, 1 H, C5-H), 4.34-4.30 (m, 1 H), 4.05-4.01 (m, 1 H), 3.88 (dd, *J* = 10.3, 6.8 Hz, 1 H, C6-H), 3.81 (dd, *J* = 10.3, 5.1 Hz, 1 H, C6-H); ^{13}C NMR (100 MHz) δ 149.2, 144.9, 142.6, 138.8, 1386, 138.4, 128.6, 128.5, 128.4, 128.0, 127.92, 127.89, 127.8, 127.7, 111.4, 107.6, 95.2, 76.5, 73.6, 73.4, 71.5, 71.1, 71.0, 68.4.



4.252

6-Furan-2-yl-3,4-dimethoxy-2-methoxymethyl-3,4-dihydro-2H-pyran (4.252). (**4-291-1**): Glucal **4.252** was prepared in 64% yield from 2-deoxyglucolactone **4.236** (61 mg, 0.3 mmol) as thick oil according to the general procedure: ^{1}H NMR (250 MHz) δ 7.33 (dd, *J* = 1.6, 0.7 Hz, 1 H), 6.50 (d, *J* = 2.0 Hz, 1 H), 6.35 (dd, *J* = 3.4, 1.9 Hz, 1 H), 5.41 (d, *J* =3.4 Hz, 1 H), 4.14-4.05 (m, 1 H), 4.00 (dd, *J* = 5.7, 3.54 Hz, 1 H), 3.73-3.68 (comp, 2 H), 3.58-3.50 (m, 1 H), 3.53 (s, 3 H), 3.43 (s, 3 H), 3.41 (s, 3 H); ^{13}C NMR (75 MHz) δ 148.8, 145.3, 142.5, 111.1, 107.6, 94.5, 76.9, 75.7, 70.6, 59.3, 59.1, 55.7; IR

(CH_2Cl_2) 2930, 2823, 1668, 1566, 1106 cm^{-1}; MS (CI) *m/z* 255.1223 [$C_{13}H_{19}O_5$ (M+1) requires 155.1232], 223 (base).

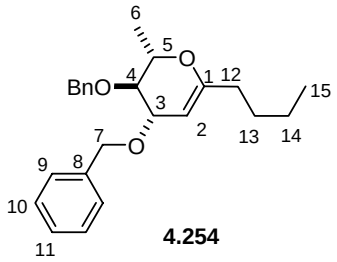


4.253

(2*S*, 3*S*, 4*S*)-3,4-Bis(benzyloxy)-6-ethyl-3,4-dihydro-2-methyl-2H-pyran (4.253). (5-24). 6-Deoxyglucal **4.253** was obtained as a clear oil in 19% yield from **4.246** (57 mg, 0.174 mmol) and EtMgCl (0.36 mmol, 2 M) after purification by chromatography eluting with Et_2O/hexanes (1:8): ^{1}H NMR (500 MHz) δ 7.37-7.25 (comp, 10 H), 4.87 (d, *J* = 11.4 Hz, 1 H), 4.70 (d, *J* = 11.4 Hz, 1 H), 4.65 (d, *J* = 11.7 Hz, 1 H), 4.57 (d, *J* = 11.7 Hz, 1 H), 4.67-4.64 (dt, *J* = 2.4, 1.0 Hz, 1 H), 4.21-4.18 (m, 1 H), 3.94 (dq, *J* = 8.8, 6.5 Hz, 1 H), 3.45 (dd, *J* = 6.4, 8.8 Hz, 1 H), 2.05 (qt, *J* = 7.4, 1.0 Hz,1 H), 1.37 (d, *J* = 6.5 Hz, 3 H), 1.04 (t, *J* = 7.5 Hz, 3 H); ^{13}C NMR (100 MHz) δ 157.7, 138.6, 138.4, 128.4, 128.0, 127.8, 127.7, 127.6, 94.4, 79.7, 77.1, 73.8, 73.7, 70.4, 26.5, 17.6, 11.2.

NMR assignments. ^{1}H NMR (500 MHz) δ 7.37-7.25 (comp, 10 H, aromatic H), 4.87 (d, *J* = 11.4 Hz, 1 H, C8-H), 4.70 (d, *J* = 11.4 Hz, 1 H, C8-H), 4.65 (d, *J* = 11.7 Hz, 1 H, C7-H), 4.57 (d, *J* = 11.7 Hz, 1 H, C7-H), 4.67-4.64 (dt, *J* = 2.4, 1.0 Hz, 1 H, C2-H), 4.21-4.18 (m, 1 H, C3-H), 3.94 (dq, *J* = 8.8, 6.5 Hz, 1 H, C5-H), 3.45 (dd, *J* = 6.4, 8.8 Hz, 1 H, C4-H), 2.05 (qt, *J* = 7.4, 1.0 Hz, 1 H, C9-H), 1.37 (d, *J* = 6.5 Hz, 3 H, C6-H),

1.04 (t, *J* = 7.5 Hz, 3 H, C10-H); ^{13}C NMR (100 MHz) δ 157.7 (C1), 138.6, 138.4, 128.4, 128.0, 127.8, 127.7, 127.6, 94.4 (C2), 79.7 (C4), 77.1 (C3), 73.8 (C8 or C5), 73.7 (C8 or C5), 70.4(C7), 26.5 (C9), 17.6 (C6), 11.2 (C10).

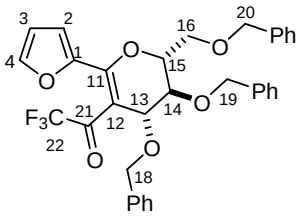


4.254

(2*S*, 3*S*, 4*S*)-3,4-Bis(benzyloxy)-6-butyl-3,4-dihydro-2-methyl-2H-pyran (4.254). (5-33). 6-Deoxyglucal **4.254** was obtained as a clear oil in 36% yield from **4.246** (47 mg, 0.144 mmol) and *n*-BuLi (0.173 mmol) after purification by chromatography eluting with Et_2O/hexanes (1:7): ^{1}H NMR (400 MHz) δ 7.39-7.26 (comp, 10 H), 4.88 (d, *J* = 11.6 Hz, 1 H), 4.70 (d, *J* = 11.3 Hz, 1 H), 4.66 (d, *J* = 11.6 Hz, 1 H), 4.60 (d, *J* = 2.4 Hz, 1 H), 4.57 (d, *J* = 11.9 Hz, 1 H), 4.23-4.17 (m, 1 H), 3.94 (dq, *J* = 8.7, 6.3 Hz, 1 H), 3.45 (dd, *J* = 8.7, 6.2 Hz, 1 H), 2.03 (t, *J* = 7.5 Hz, 2 H), 1.50-1.19 (comp, 4 H), 1.38 (d, *J* = 6.3 Hz, 3 H), 0.90 (t, *J* = 7.3 Hz, 3 H); ^{13}C NMR (100 MHz) δ 156.6, 138.6, 138.4, 128.4, 127.9, 127.7, 127.6, 127.5, 95.0, 79.6, 77.1, 73.8, 73.7, 70.3, 33.2, 28.9, 22.3, 17.5, 13.9; IR ($CHCl_3$) 2956, 1673, 1453, 1103 cm^{-1}; MS (CI) *m/z* 367 (base), 259.

NMR assignments. **1**H NMR (400 MHz) δ 7.39-7.26 (comp, 10 H, aromatic H), 4.88 (d, *J* = 11.6 Hz, 1 H, C7-H), 4.70 (d, *J* = 11.3 Hz, 1 H, C7-H), 4.66 (d, *J* = 11.6 Hz, 1 H, C7-H), 4.60 (d, *J* = 2.4 Hz, 1 H, C2-H), 4.57 (d, *J* = 11.9 Hz, 1 H, C7-H), 4.23-4.17

(m, 1 H, C3-H), 3.94 (dq, *J* = 8.7, 6.3 Hz, 1 H, C5-H), 3.45 (dd, *J* = 8.7, 6.2 Hz, 1 H, C4-H), 2.03 (t, *J* = 7.5 Hz, 2 H, C12-H), 1.50-1.19 (comp, 4 H, C13-H & C14-H), 1.38 (d, *J* = 6.3 Hz, 3 H, C6-H), 0.90 (t, *J* = 7.3 Hz, 3 H, C15-H); ^{13}C NMR (100 MHz) δ 156.6 (C1), 138.6, 138.4, 128.4, 127.9, 127.7, 127.6, 127.5, 95.0 (C2), 79.6 (C4), 77.1 (C3), 73.8, 73.7, 70.3 (C7), 33.2 (C12), 28.9 (C13), 22.3 (C14), 17.5 (C6), 13.9 (C15).



4.235

1-(4,5-Bis-benzyloxy-6-benzyloxymethyl-2-furan-2-yl-5,6-dihydro-4H-pyran-3-yl)-2,2,2-trifluoro-ethanone (4.235). (4-269). Trifluoroketone **4.235** was prepared in 63% yield from furylglucal **4.222** (13.3 mg, 0.0276 mmol), pyridine (21 μL, 0.266 mmol), DMAP (12 mg, 0.098 mmol) and TFAA (74 mg, 0.35 mmol) in THF (1.3 mL) as clear oil according to a slightly modified general procedure B: ^{1}H NMR (400 MHz) δ 7.51 (d, *J* = 1.0 Hz, 1 H), 7.40-7.24 (comp, 13 H), 7.19-7.14 (comp, 2 H), 6.85 (d, *J* = 3.4 Hz, 1 H), 6.51 (dd, *J* = 3.4, 1.7 Hz, 1 H), 4.80 (dq, *J* = 9.5, 2.1 Hz, 1 H), 4.62 (d, *J* = 12.0 Hz, 1 H), 4.59 (d, *J* = 12.0 Hz, 1 H), 4.58 (m, 1 H), 4.50 (s, 1 H), 4.48 (d, *J* = 11.3 Hz, 1 H), 4.42 (d, *J* = 11.3 Hz, 1 H), 3.96 (app t, *J* = 2.2 Hz, 1 H), 3.87 (dd, *J* = 10.5, 7.2 Hz, 1 H), 3.71 (dd, *J* = 10.5, 5.1 Hz, 1 H); ^{13}C NMR (100 MHz) δ 186.7, 186.5, 151.4, 147.3, 145.3, 137.7, 137.4, 137.2, 128.6, 128.4, 128.3, 128.1, 127.9, 127.84, 127.82, 127.7, 127.5, 117.3, 113.8, 112.3, 106.0, 77.4, 77.3, 77.0, 76.7, 73.3, 72.3, 71.9, 71.5, 66.7; IR ($CHCl_3$) 2865, 1696, 1630, 1144 cm^{-1}.

References

1) Lee, S. B.; Rhee, S. G., "Significance of Pip2 Hydrolysis and Regulation of Phospholipase-C Isozymes." *Curr. Opin. Cell Biol.* **1995**, *7*, 183-189.

2) Rhee, S. G.; Choi, K. D., "Regulation of Inositol Phospholipid-Specific Phospholipase-C Isozymes." *J. Biol. Chem.* **1992**, *267*, 12393-12396.

3) Rhee, S. G.; Suh, P. G.; Ryu, S. H.; Lee, S. Y., "Studies of Inositol Phospholipid Specific Phospholipase-C." *Science* **1989**, *244*, 546-550.

4) Nishizuka, Y., "Studies and Perspectives of Protein-Kinase-C." *Science* **1986**, *233*, 305-312.

5) Kikkawa, U.; Nishizuka, Y., "The Role of Protein-Kinase-C in Cell-Surface Signal Transduction." *J. Cell. Biochem.* **1986**, 107-107.

6) Kaibuchi, K.; Takai, Y.; Nishizuka, Y., "Cooperative Roles of Various Membrane Phospholipids in the Activation of Calcium-Activated, Phospholipid-Dependent Protein-Kinase." *J. Biol. Chem.* **1981**, *256*, 7146-7149.

7) Nishizuka, Y., "Intracellular Signaling by Hydrolysis of Phospholipids and Activation of Protein-Kinase-C." *Science* **1992**, *258*, 607-614.

8) Liscovitch, M., "Crosstalk among Multiple Signal-Activated Phospholipases." *Trends Biochem. Sci.* **1992**, *17*, 393-399.

9) Kraft, A. S.; Anderson, W. B., "Phorbol Esters Increase the Amount of Ca-2+, Phospholipid-Dependent Protein-Kinase Associated with Plasma-Membrane." *Nature* **1983**, *301*, 621-623.

10) Aihara, H.; Asaoka, Y.; Yoshida, K.; Nishizuka, Y., "Sustained Activation of Protein-Kinase-C Is Essential to Hl-60 Cell-Differentiation to Macrophage." *Proc. Natl. Acad. Sci. U. S. A.* **1991**, *88*, 11062-11066.

11) Asaoka, Y.; Oka, M.; Yoshida, K.; Nishizuka, Y., "Metabolic-Rate of Membrane-Permeant Diacylglycerol and Its Relation to Human Resting Lymphocyte-T Activation." *Proc. Natl. Acad. Sci. U. S. A.* **1991**, *88*, 8681-8685.

12) Georgieva, D. N.; Perbandt, M.; Rypniewski, W.; Hristov, K.; Genov, N.; Betzel, C., "The X-ray structure of a snake venom Gln48 phospholipase A2 at 1.9 .ANG. resolution reveals anion-binding sites." *Biochem. Biophys. Res. Commun.* **2004**, *316*, 33-38.

13) Jasti, J.; Paramasivam, M.; Srinivasan, A.; Singh, T. P., "Structure of an acidic phospholipase A2 from Indian saw-scaled viper (Echis carinatus) at 2.6 .ANG. resolution

reveals a novel intermolecular interaction." *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **2004**, *D60*, 66-72.

14) Ketelhut, D. F. J.; Homem de Mello, M.; Veronese, E. L. G.; Esmeraldino, L. E.; Murakami, M. T.; Arni, R. K.; Giglio, J. R.; Cintra, A. C. O.; Sampaio, S. V., "Isolation, characterization and biological activity of acidic phospholipase A2 isoforms from Bothrops jararacussu snake venom." *Biochimie* **2003**, *85*, 983-991.

15) Magro, A. J.; Murakami, M. T.; Marcussi, S.; Soares, A. M.; Arni, R. K.; Fontes, M. R. M., "Crystal structure of an acidic platelet aggregation inhibitor and hypotensive phospholipase A2 in the monomeric and dimeric states: insights into its oligomeric state." *Biochem. Biophys. Res. Commun.* **2004**, *323*, 24-31.

16) Takeda, A. A. S.; Dos Santos, J. I.; Marcussi, S.; Silveira, L. B.; Soares, A. M.; Fontes, M. R. M., "Crystallization and preliminary x-ray diffraction analysis of an acidic phospholipase A2 complexed with p-bromophenacyl bromide and a-tocopherol inhibitors at 1.9- and 1.45-.ANG. resolution." *Biochim. Biophys. Acta* **2004**, *1699*, 281-284.

17) Dijkstra, B. W.; Kalk, K. H.; Drenth, J.; de Haas, G. H.; Egmond, M. R.; Slotboom, A. J., "Role of the N-terminus in the interaction of pancreatic phospholipase A2 with aggregated substrates. Properties and crystal structure of transaminated phospholipase A2." *Biochemistry* **1984**, *23*, 2759-66.

18) Pan Ying, H.; Yu, B.-Z.; Berg Otto, G.; Jain Mahendra, K.; Bahnson Brian, J., "Crystal structure of phospholipase A2 complex with the hydrolysis products of platelet activating factor: equilibrium binding of fatty acid and lysophospholipid-ether at the active site may be mutually exclusive." *Biochemistry* **2002**, *41*, 14790-800.

19) Thunnissen, M. M.; Franken, P. A.; de Haas, G. H.; Drenth, J.; Kalk, K. H.; Verheij, H. M.; Dijkstra, B. W., "Crystal structure of a porcine pancreatic phospholipase A2 mutant. A large conformational change caused by the F63V point mutation." *J. Mol. Biol.* **1993**, *232*, 839-55.

20) Edwards, S. H.; Thompson, D.; Baker, S. F.; Wood, S. P.; Wilton, D. C., "The Crystal Structure of the H48Q Active Site Mutant of Human Group IIA Secreted Phospholipase A2 at 1.5 ANG. Resolution Provides an Insight into the Catalytic Mechanism." *Biochemistry* **2002**, *41*, 15468-15476.

21) Pan, Y. H.; Yu, B.-Z.; Singer, A. G.; Ghomashchi, F.; Lambeau, G.; Gelb, M. H.; Jain, M. K.; Bahnson, B. J., "Crystal structure of human group X secreted phospholipase A2. Electrostatically neutral interfacial binding surface targets zwitterionic membranes." *J. Biol. Chem.* **2002**, *277*, 29086-29093.

22) Church, W. B.; Lei, P.-W.; Ogg, D. J.; Scott, K. F., "Crystallization and preliminary X-ray diffraction studies of a new crystal form of human secretory type IIA phospholipase A2." *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **2000**, *D56*, 1482-1484.

23) Clark, J. D.; Lin, L. L.; Kriz, R. W.; Ramesha, C. S.; Sultzman, L. A.; Lin, A. Y.; Milona, N.; Knopf, J. L., "A novel arachidonic acid-selective cytosolic PLA2 contains a Ca(2+)-dependent translocation domain with homology to PKC and GAP." *Cell* **1991**, *65*, 1043-51.

24) Leslie, C. C., "Kinetic properties of a high molecular mass arachidonoyl-hydrolyzing phospholipase A2 that exhibits lysophospholipase activity." *J. Biol. Chem.* **1991**, *266*, 11366-71.

25) Clark, J. D.; Milona, N.; Knopf, J. L., "Purification of a 110-kilodalton cytosolic phospholipase A2 from the human monocytic cell line U937." *Proc. Natl. Acad. Sci. U. S. A.* **1990**, *87*, 7708-12.

26) Diez, E.; Mong, S., "Purification of a phospholipase A2 from human monocytic leukemic U937 cells. Calcium-dependent activation and membrane association." *J. Biol. Chem.* **1990**, *265*, 14654-61.

27) Chiba, H.; Michibata, H.; Wakimoto, K.; Seishima, M.; Kawasaki, S.; Okubo, K.; Mitsui, H.; Torii, H.; Imai, Y., "Cloning of a Gene for a Novel Epithelium-specific Cytosolic Phospholipase A2, cPLA2d, Induced in Psoriatic Skin." *J. Biol. Chem.* **2004**, *279*, 12890-12897.

28) Hillman, J. L.; Bandman, O.; Guegler, K. J.; Corley, N. C.; Baughn, M. R.; Azimzai, Y.; Lal, P.; Lu, D. A. M. "Cloning, sequence, expression and therapeutic use of human phospholipases." 99-US25021, 2000024911, 19991027., 2000.

29) Kriz, R.; Song, C. "Cloning and characterization of human calcium-independent cytosolic phospholipase A2-b and its use for screening anti-inflammatory compounds." 99-460145, 6287838, 19991213., 2001.

30) Ma, Z.; Ramanadham, S.; Hu, Z.; Turk, J., "Cloning and expression of a group IV cytosolic Ca2+-dependent phospholipase A2 from rat pancreatic islets. Comparison of the expressed activity with that of an islet group VI cytosolic Ca2+-independent phospholipase A2." *Biochim. Biophys. Acta* **1998**, *1391*, 384-400.

31) Pickard, R. T.; Strifler, B. A.; Kramer, R. M.; Sharp, J. D., "Molecular cloning of two new human paralogs of 85-kDa cytosolic phospholipase A2." *J. Biol. Chem.* **1999**, *274*, 8823-8831.

32) Dessen, A.; Tang, J.; Schmidt, H.; Stahl, M.; Clark, J. D.; Seehra, J.; Somers, W. S., "Crystal structure of human cytosolic phospholipase A2 reveals a novel topology and catalytic mechanism." *Cell* **1999**, *97*, 349-360.

33) Perisic, O.; Fong, S.; Lynch, D. E.; Bycroft, M.; Williams, R. L., "Crystal structure of a calcium-phospholipid binding domain from cytosolic phospholipase A2." *J. Biol. Chem.* **1998**, *273*, 1596-1604.

34) Ghosh, S.; Strum, J. C.; Sciorra, V. A.; Daniel, L.; Bell, R. M., "Raf-1 kinase possesses distinct binding domains for phosphatidylserine and phosphatidic acid. Phosphatidic acid regulates the translocation of Raf-1 in 12-O-tetradecanoylphorbol-13-acetate-stimulated Madin-Darby canine kidney cells." *J. Biol. Chem.* **1996**, *271*, 8472-80.

35) English, D.; Cui, Y.; Siddiqui, R. A., "Messenger functions of phosphatidic acid." *Chem. Phys. Lipids* **1996**, *80*, 117-32.

36) Imagawa, W.; Bandyopadhyay, G.; Nandi, S., "Multifunctional phosphatidic acid signaling in mammary epithelial cells: stimulation of phosphoinositide hydrolysis and conversion to diglyceride." *J. Cell. Physiol.* **1995**, *163*, 561-9.

37) Wang, X. M.; Xu, L. W.; Zheng, L., "Cloning and Expression of Phosphatidylcholine-Hydrolyzing Phospholipase-D from Ricinus-Communis L." *J. Biol. Chem.* **1994**, *269*, 20312-20317.

38) Rose, K.; Rudge, S. A.; Frohman, M. A.; Morris, A. J.; Engebrecht, J. A., "Phospholipase D signaling is essential for meiosis." *Proc. Natl. Acad. Sci. U. S. A.* **1995**, *92*, 12151-12155.

39) Hammond, S. M.; Jenco, J. M.; Nakashima, S.; Cadwallader, K.; Gu, Q. M.; Cook, S.; Nozawa, Y.; Prestwich, G. D.; Frohman, M. A.; Morris, A. J., "Characterization of two alternately spliced forms of phospholipase D1 - Activation of the purified enzymes by phosphatidylinositol 4,5-bisphosphate, ADP-ribosylation factor, and RHO family monomeric GTP-binding proteins and protein kinase C-alpha." *J. Biol. Chem.* **1997**, *272*, 3860-3868.

40) Hammond, S. M.; Altshuller, Y. M.; Sung, T. C.; Rudge, S. K.; Rose, K.; Engebrecht, J.; Morris, A. J.; Frohman, M. A., "Human Adp-Ribosylation Factor-Activated Phosphatidylcholine-Specific Phospholipase-D Defines a New and Highly Conserved Gene Family." *J. Biol. Chem.* **1995**, *270*, 29640-29643.

41) Colley, W. C.; Sung, T. C.; Roll, R.; Jenco, J.; Hammond, S. M.; Altshuller, Y.; BarSagi, D.; Morris, A. J.; Frohman, M. A., "Phospholipase D2, a distinct phospholipase D isoform with novel regulatory properties that provokes cytoskeletal reorganization." *Curr. Biol.* **1997**, *7*, 191-201.

42) Abergel, C.; Abousalham, A.; Chenivesse, S.; Riviere, M.; Moustacas-Gardies, A. M.; Verger, R., "Crystallization and preliminary crystallographic study of a recombinant phospholipase D from cowpea (Vigna unguiculata L. Walp)." *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **2001**, *D57*, 320-322.

43) Leiros, I.; Secundo, F.; Zambonelli, C.; Servi, S.; Hough, E., "The first crystal structure of a phospholipase D." *Structure* **2000**, *8*, 655-667.

44) Leiros, I.; Hough, E.; D'Arrigo, P.; Carrea, G.; Pedrocchi-Fantoni, G.; Secundo, F.; Servi, S., "Crystallization and preliminary x-ray diffraction studies of phospholipase D from Streptomyces sp." *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **2000**, *D56*, 466-468.

45) Stuckey, J. A.; Dixon, J. E., "Crystal structure of a phospholipase D family member." *Nature Structural Biology* **1999**, *6*, 278-284.

46) Suzuki, A.; Kakuno, K.; Iwasaki, Y.; Yamane, T.; Yamane, T., "Crystallization and preliminary X-ray diffraction studies of phospholipase D from Streptomyces antibioticus." *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **1999**, *D55*, 317-319.

47) Zhao, Y.; Stuckey, J. A.; Lohse, D. L.; Dixon, J. E., "Expression, characterization, and crystallization of a member of the novel phospholipase D family of phosphodiesterases." *Protein Sci.* **1997**, *6*, 2655-2658.

48) Davies, D. R.; Interthal, H.; Champoux, J. J.; Hol, W. G. J., "Crystal structure of a transition state mimic for Tdp1 assembled from vanadate, DNA, and a topoisomerase I-derived peptide." *Chem. Biol.* **2003**, *10*, 139-147.

49) Davies, D. R.; Interthal, H.; Champoux, J. J.; Hol, W. G. J., "The crystal structure of human tyrosyl-DNA phosphodiesterase, Tdp1." *Structure* **2002**, *10*, 237-248.

50) Heinz, D. W.; Ryan, M.; Smith, M. P.; Weaver, L. H.; Keana, J. F. W.; Griffith, O. H., "Crystal structure of phosphatidylinositol-specific phospholipase C from Bacillus cereus in complex with glucosaminyl(alpha 1->6)-D-myo-inositol, an essential fragment of GPI anchors." *Biochemistry* **1996**, *35*, 9496-9504.

51) Essen, L. O.; Perisic, O.; Katan, M.; Wu, Y. Q.; Roberts, M. F.; Williams, R. L., "Structural mapping of the catalytic mechanism for a mammalian phosphoinositide-specific phospholipase C." *Biochemistry* **1997**, *36*, 1704-1718.

52) Grobler, J. A.; Essen, L. O.; Williams, R. L.; Hurley, J. H., "C2 domain conformational changes in phospholipase C-delta 1." *Nature Structural Biology* **1996**, *3*, 788-795.

53) Essen, L.-O.; Perisic, O.; Cheung, R.; Katan, M.; Williams, R. L., "Crystal structure of a mammalian phosphoinositide-specific phospholipase C.delta." *Nature* **1996**, *380*, 595-602.

54) Wolf, R. A.; Gross, R. W., "Identification of Neutral Active Phospholipase-C Which Hydrolyzes Choline Glycerophospholipids and Plasmalogen Selective Phospholipase-A2 in Canine Myocardium." *J. Biol. Chem.* **1985**, *260*, 7295-7303.

55) Sheikhnejad, R. G.; Srivastava, P. N., "Isolation and Properties of a Phosphatidylcholine-Specific Phospholipase-C from Bull Seminal Plasma." *J. Biol. Chem.* **1986**, *261*, 7544-7549.

56) Clark, M. A.; Shorr, R. G. L.; Bomalski, J. S., "Antibodies Prepared to Bacillus-Cereus Phospholipase-C Cross-React with a Phosphatidylcholine Preferring Phospholipase-C in Mammalian-Cells." *Biochem. Biophys. Res. Commun.* **1986**, *140*, 114-119.

57) Titball, R. W., "Bacterial Phospholipases-C." *Microbiological Reviews* **1993**, *57*, 347-366.

58) Little, C.; Aurebekk, B.; Otnaess, A. B., "Purification by Affinity Chromatography of Phospholipase-C from Bacillus-Cereus." *FEBS Lett.* **1975**, *52*, 175-179.

59) Takahashi, T.; Sugahara, T.; Ohsaka, A., "Phospholipase C from Clostridium perfringens." *Methods Enzymol.* **1981**, *71 Pt C*, 710-25.

60) Takahashi, T.; Sugahara, T.; Osaka, A., "Purification of alpha-toxin (phospholipase C) of Clostridium perfringens by affinity chromatography." *Japanese Journal of Medical Science & Biology* **1974**, *27*, 89-92.

61) Takahashi, T.; Sugahara, T.; Ohsaka, A., "Purification of Clostridium perfringens phospholipase C (alpha-toxin) by affinity chromatography on agarose-linked egg-yolk lipoprotein." *Biochim. Biophys. Acta* **1974**, *351*, 155-71.

62) Berka, R. M.; Gray, G. L.; Vasil, M. L., "Studies of phospholipase C (heat-labile hemolysin) in Pseudomonas aeruginosa." *Infect. Immun.* **1981**, *34*, 1071-4.

63) Okabe, A.; Shimizu, T.; Hayashi, H., "Cloning and Sequencing of a Phospholipase-C Gene of Clostridium-Perfringens." *Biochem. Biophys. Res. Commun.* **1989**, *160*, 33-39.

64) Tso, J. Y.; Siebel, C., "Cloning and Expression of the Phospholipase-C Gene from Clostridium-Perfringens and Clostridium-Bifermentans." *Infect. Immun.* **1989**, *57*, 468-476.

65) Hunter, S. E. C.; Clarke, I. N.; Kelly, D. C.; Titball, R. W., "Cloning and Nucleotide Sequencing of the Clostridium-Perfringens Epsilon-Toxin Gene and Its Expression in Escherichia-Coli." *Infect. Immun.* **1992**, *60*, 102-110.

66) Johansen, T.; Holm, T.; Guddal, P. H.; Sletten, K.; Haugli, F. B.; Little, C., "Cloning and Sequencing of the Gene Encoding the Phosphatidylcholine-Preferring Phospholipase-C of Bacillus-Cereus." *Gene* **1988**, *65*, 293-304.

67) Projan, S. J.; Kornblum, J.; Kreiswirth, B.; Moghazeh, S. L.; Eisner, W.; Novick, R. P., "Nucleotide-Sequence - the Beta-Hemolysin Gene of Staphylococcus-Aureus." *Nucleic Acids Res.* **1989**, *17*, 3305-3305.

68) Shen, B. F.; Tai, P. C.; Pritchard, A. E.; Vasil, M. L., "Nucleotide-Sequences and Expression in Escherichia-Coli of the in-Phase Overlapping Pseudomonas-Aeruginosa Plcr Genes." *J. Bacteriol.* **1987**, *169*, 4602-4607.

69) Leslie, D.; Fairweather, N.; Pickard, D.; Dougan, G.; Kehoe, M., "Phospholipase-C and Hemolytic Activities of Clostridium-Perfringens Alpha-Toxin Cloned in Escherichia-Coli - Sequence and Homology with a Bacillus-Cereus Phospholipase-C." *Mol. Microbiol.* **1989**, *3*, 383-392.

70) Zwaal, R. F. A.; Roelofsen, B.; Comfurius, P.; Van Deenen, L. L. M., "Complete purification and some properties of phospholipase C from Bacillus cereus." *Biochim. Biophys. Acta* **1971**, *233*, 474-9.

71) Otnaess, A. B.; Holm, T., "The effect of phospholipase C on human blood platelets." *J. Clin. Invest.* **1976**, *57*, 1419-25.

72) Levine, L.; Xiao, D. M.; Little, C., "Increased arachidonic acid metabolites from cells in culture after treatment with the phosphatidylcholine-hydrolyzing phospholipase C from Bacillus cereus." *Prostaglandins* **1987**, *34*, 633-42.

73) Johansen, T.; Bjorkoy, G.; Overvatn, A.; Diazmeco, M. T.; Traavik, T.; Moscat, J., "NIH 3t3 Cells Stably Transfected with the Gene Encoding Phosphatidylcholine-Hydrolyzing Phospholipase-C from Bacillus-Cereus Acquire a Transformed Phenotype." *Mol. Cell. Biol.* **1994**, *14*, 646-654.

74) Diazlaviada, I.; Larrodera, P.; Diazmeco, M. T.; Cornet, M. E.; Guddal, P. H.; Johansen, T.; Moscat, J., "Evidence for a Role of Phosphatidylcholine-Hydrolyzing Phospholipase-C in the Regulation of Protein-Kinase-C by Ras and Src Oncogenes." *EMBO J.* **1990**, *9*, 3907-3912.

75) Larrodera, P.; Cornet, M. E.; Diazmeco, M. T.; Lopezbarahona, M.; Diazlaviada, I.; Guddal, P. H.; Johansen, T.; Moscat, J., "Phospholipase-C Mediated Hydrolysis of Phosphatidylcholine Is an Important Step in Pdgf-Stimulated DNA-Synthesis." *Cell* **1990**, *61*, 1113-1120.

76) Hergenrother, P. J.; Martin, S. F., "Determination of the kinetic parameters for phospholipase C (Bacillus cereus) on different phospholipid substrates using a chromogenic assay based on the quantitation of inorganic phosphate." *Anal. Biochem.* **1997**, *251*, 45-49.

77) Hough, E.; Hansen, L. K.; Birknes, B.; Jynge, K.; Hansen, S.; Hordvik, A.; Little, C.; Dodson, E.; Derewenda, Z., "High-resolution (1.5 .ANG.) crystal structure of phospholipase C from Bacillus cereus." *Nature* **1989**, *338*, 357-60.

78) Little, C., "The histidine residues of phospholipase C from Bacillus cereus." *Biochem. J.* **1977**, *167*, 399-404.

79) Otnaess, A. B.; Little, C.; Sletten, K.; Wallin, R.; Johnsen, S.; Flengsrud, R.; Prydz, H., "Some characteristics of phospholipase C from Bacillus cereus." *Eur. J. Biochem.* **1977**, *79*, 459-68.

80) Hansen, S.; Hough, E.; Svensson, L. A.; Wong, Y. L.; Martin, S. F., "Crystal structure of phospholipase C from Bacillus cereus complexed with a substrate analog." *J. Mol. Biol.* **1993**, *234*, 179-87.

81) Hansen, S.; Hansen, L. K.; Hough, E., "The crystal structure of Tris-inhibited phospholipase C from Bacillus cereus at 1.9 .ANG. resolution. The nature of the metal ion in site 2." *J. Mol. Biol.* **1993**, *231*, 870-6.

82) Hansen, S.; Hansen, L. K.; Hough, E., "Crystal structures of phosphate, iodide and iodate-inhibited phospholipase C from Bacillus cereus and structural investigations of the binding of reaction products and a substrate analog." *J. Mol. Biol.* **1992**, *225*, 543-9.

83) Kim, E. E.; Wyckoff, H. W., "Structure of Alkaline-Phosphatases." *Clin. Chim. Acta* **1990**, *186*, 175-187.

84) Sowadski, J. M.; Handschumacher, M. D.; Murthy, H. M.; Foster, B. A.; Wyckoff, H. W., "Refined structure of alkaline phosphatase from Escherichia coli at 2.8 A resolution." *J. Mol. Biol.* **1985**, *186*, 417-33.

85) Suck, D.; Dominguez, R.; Lahm, A.; Volbeda, A., "The 3-Dimensional Structures of Penicillium-P1 and Aspergillus-S1 Nuclease." *J. Cell. Biochem.* **1993**, 154-154.

86) Volbeda, A.; Lahm, A.; Sakiyama, F.; Suck, D., "Crystal-Structure of Penicillium-Citrinum P1 Nuclease at 2.8-a Resolution." *EMBO J.* **1991**, *10*, 1607-1618.

87) Martin, S. F.; Wong, Y.-L.; Wagman, A. S., "Design, Synthesis, and Evaluation of Phospholipid Analogs as Inhibitors of the Bacterial Phospholipase C from Bacillus cereus." *J. Org. Chem.* **1994**, *59*, 4821-31.

88) Wilson, I. B.; Dayan, J., "The Free Energy of Hydrolysis of Phosphoryl-Phosphatase." *Biochemistry* **1965**, *43*, 645-9.

89) Holtz, K. M.; Kantrowitz, E. R., "The mechanism of the alkaline phosphatase reaction: insights from NMR, crystallography and site-specific mutagenesis." *FEBS Lett.* **1999**, *462*, 7-11.

90) Jones, S. R.; Kindman, L. A.; Knowles, J. R., "Stereochemistry of Phosphoryl Group Transfer Using a Chiral O-16, O-17, O-18 Stereochemical Course of Alkaline-Phosphatase." *Nature* **1978**, *275*, 564-565.

91) Fujimoto, M.; Kuninaka, A.; Yoshino, H., "Nuclease from Penicillium citrinum. II. Identity of phosphodiesterase and phosphomonoesterase activities with nuclease P1 (a nuclease from Penicillium citrinum)." *Agricultural and Biological Chemistry* **1974**, *38*, 785-90.

92) Fujimoto, M.; Kuninaka, A.; Yoshino, H., "Nuclease from Penicillium citrinum. V. Physical and chemical properties of nuclease P1." *Agricultural and Biological Chemistry* **1975**, *39*, 1991-7.

93) Lahm, A.; Volbeda, A.; Suck, D., "Crystallization and Preliminary Crystallographic Analysis of P1 Nuclease from Penicillium-Citrinum." *J. Mol. Biol.* **1990**, *215*, 207-210.

94) Sundell, S.; Hansen, S.; Hough, E., "A Proposal for the Catalytic Mechanism in Phospholipase-C Based on Interaction Energy and Distance Geometry Calculations." *Protein Eng.* **1994**, *7*, 571-577.

95) Steitz, T. A.; Steitz, J. A., "A General 2-Metal-Ion Mechanism for Catalytic Rna." *Proc. Natl. Acad. Sci. U. S. A.* **1993**, *90*, 6498-6502.

96) Romier, C.; Dominguez, R.; Lahm, A.; Dahl, O.; Suck, D., "Recognition of single-stranded DNA by nuclease P1: high resolution crystal structures of complexes with substrate analogs." *Proteins: Struct., Funct., Genet.* **1998**, *32*, 414-424.

97) Dougherty, D. A., "Cation-pi interactions in chemistry and biology: A new view of benzene, Phe, Tyr, and Trp." *Science* **1996**, *271*, 163-168.

98) Roderick, S. L.; Chan, W. W.; Agate, D. S.; Olsen, L. R.; Vetting, M. W.; Rajashankar, K. R.; Cohen, D. E., "Structure of human phosphatidylcholine transfer protein in complex with its ligand." *Nature Structural Biology* **2002**, *9*, 507-511.

99) Cametti, M.; Nissinen, M.; Dalla Cort, A.; Mandolini, L.; Rissanen, K., "Recognition of Alkali Metal Halide Contact Ion Pairs by Uranyl-Salophen Receptors Bearing Aromatic Sidearms. The Role of Cation-p Interactions." *J. Am. Chem. Soc.* **2005**, *127*, 3831-3837.

100) Soerme, P.; Arnoux, P.; Kahl-Knutsson, B.; Leffler, H.; Rini, J. M.; Nilsson, U. J., "Structural and Thermodynamic Studies on Cation-P Interactions in Lectin-Ligand

Complexes: High-Affinity Galectin-3 Inhibitors through Fine-Tuning of an Arginine-Arene Interaction." *J. Am. Chem. Soc.* **2005**, *127*, 1737-1743.

101) Martin, S. F.; Hergenrother, P. J., "Enzymatic synthesis of a modified phospholipid and its evaluation as a substrate for B-cereus phospholipase C." *Bioorg. Med. Chem. Lett.* **1998**, *8*, 593-596.

102) Strater, N.; Lipscomb, W. N.; Klabunde, T.; Krebs, B., "Two-metal ion catalysis in enzymatic acyl- and phosphoryl-transfer reactions." *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2024-2055.

103) Martin, S. F.; Hergenrother, P. J., "Catalytic cycle of the phosphatidylcholine-preferring phospholipase C from Bacillus cereus. Solvent viscosity, deuterium isotope effects, and proton inventory studies." *Biochemistry* **1999**, *38*, 4403-4408.

104) El-Sayed, M. Y.; Roberts, M. F., "Charged detergents enhance the activity of phospholipase C (Bacillus cereus) towards micellar short-chain phosphatidylcholine." *Biochim. Biophys. Acta* **1985**, *831*, 133-41.

105) Martin, S. F.; Hergenrother, P. J., "General base catalysis by the phosphatidylcholine-preferring phospholipase C from Bacillus cereus: The role of Glu4 and Asp55." *Biochemistry* **1998**, *37*, 5755-5760.

106) Antikainen, N. M.; Monzingo, A. F.; Franklin, C. L.; Robertus, J. D.; Martin, S. F., "Using x-ray crystallography of the Asp55Asn mutant of the phosphatidylcholine-preferring phospholipase C from Bacillus cereus to support the mechanistic role of Asp55 as the general base." *Arch. Biochem. Biophys.* **2003**, *417*, 81-86.

107) Eibl, H., "Synthesis of Glycerophospholipids." *Chem. Phys. Lipids* **1980**, *26*, 405-429.

108) Slotin, L. A., "Current Methods of Phosphorylation of Biological Molecules." *Synthesis* **1977**, 737-752.

109) Hajdu, J., "Recent advances in phospholipid synthesis." *Recent Research Developments in Lipids Research* **1999**, *3*, 165-189.

110) Ramirez, F.; Marecek, J. F., "Synthesis of Phosphodiesters - the Cyclic Enediol Phosphoryl (Cep) Method." *Synthesis* **1985**, 449-488.

111) Roodsari, F. S.; Wu, D.; Pum, G. S.; Hajdu, J., "A New Approach to the Stereospecific Synthesis of Phospholipids. The Use of L-Glyceric Acid for the Preparation of Diacylglycerols, Phosphatidylcholines, and Related Derivatives." *J. Org. Chem.* **1999**, *64*, 7727-7737.

112) Baer, E.; Kates, M., "Synthesis of enantiomeric a-lecithins." *J. Am. Chem. Soc.* **1950**, *72*, 942-9.

113) Lamant, V.; Chap, H.; Klaebe, A.; Perie, J. J.; Willson, M., "Synthesis of a Thiophospho Analog of Platelet-Activating-Factor (Rs)-1-Hexadecyl-2-Acetylglycero-3-Thiophosphocholine and (S)-1-Hexadecyl-2-Acetylglycero-3-Thiophosphocholine." *J. Chem. Soc., Chem. Commun.* **1987**, 1608-1609.

114) Martin, S. F.; Josey, J. A., "A general protocol for the preparation of phospholipids via phosphite coupling." *Tetrahedron Lett.* **1988**, *29*, 3631-4.

115) Eibl, H., "Phospholipid Synthesis - Oxazaphospholanes and Dioxaphospholanes as Intermediates." *Proc. Natl. Acad. Sci. U. S. A.* **1978**, *75*, 4074-4077.

116) Murakami, K.; Molitor, E. J.; Liu, H. W., "An efficient synthesis of unsymmetrical optically active phosphatidyl glycerol." *J. Org. Chem.* **1999**, *64*, 648-651.

117) Schmid, C. R.; Bryant, J. D.; Dowlatzedah, M.; Phillips, J. L.; Prather, D. E.; Schantz, R. D.; Sear, N. L.; Vianco, C. S., "Synthesis of 2,3-O-isopropylidene-D-glyceraldehyde in high chemical and optical purity: observations on the development of a practical bulk process." *J. Org. Chem.* **1991**, *56*, 4056-8.

118) Burgos, C. E.; Ayer, D. E.; Johnson, R. A., "A New, Asymmetric-Synthesis of Lipids and Phospholipids." *J. Org. Chem.* **1987**, *52*, 4973-4977.

119) Ali, S.; Bittman, R., "Facile Diacylation of Glycidyl Tosylate - Chiral Synthesis of Symmetric-Chain Glycerophospholipids." *J. Org. Chem.* **1988**, *53*, 5547-5549.

120) Guivisdalsky, P. N.; Bittman, R., "Regiospecific Opening of Glycidyl Derivatives Mediated by Boron-Trifluoride - Asymmetric-Synthesis of Ether-Linked Phospholipids." *J. Org. Chem.* **1989**, *54*, 4637-4642.

121) Phuong, N. H.; Thuong, N. T.; Chabrier, P., "New Method for Preparation of Glycollecithines and Lecithines." *Comptes Rendus Hebdomadaires Des Seances De L Academie Des Sciences Serie C* **1976**, *283*, 229-231.

122) Gadek, T. R., "Trimethylsilyl Triflate Mediated Introduction of Phospholipid Head Groups." *Tetrahedron Lett.* **1989**, *30*, 915-918.

123) Michalski, J.; Dabkowski, W., "State of the art. Chemical synthesis of biophosphates and their analogues via p(III) derivatives." In *New Aspects in Phosphorus Chemistry Iv*, ed.; 2004; 'Vol.' 232, 93-144.

124) Martin, S. F.; Josey, J. A.; Wong, Y.-L.; Dean, D. W., "General Method for the Synthesis of Phospholipid Derivatives of 1,2-O-Diacyl-sn-Glycerols." *J. Org. Chem.* **1994**, *59*, 4805-20.

125) Nifantiev, E. E.; Grachev, M. K.; Burmistrov, S. Y., "Amides of trivalent phosphorus acids as phosphorylating reagents for proton-donating nucleophiles." *Chemical Reviews* **2000**, *100*, 3755-3799.

126) Lindberg, J.; Ekeroth, J.; Konradsson, P., "Efficient synthesis of phospholipids from glycidyl phosphates." *J. Org. Chem.* **2002**, *67*, 194-199.

127) Hebert, N.; Just, G., "Synthesis of Phospholipids Using an Inverse Phosphite Triester Approach." *J. Chem. Soc., Chem. Commun.* **1990**, 1497-1498.

128) Mlotkowska, B.; Olejnik, J., "A Synthesis of Rac-S-(2-Acetoxy-3-Hexadecyloxypropyl) Thiophosphocholine, the Isosteric and Isopolar Paf Analog." *Liebigs Annalen* **1995**, 1467-1470.

129) Martin, S. F.; Dean, D. W.; Wagman, A. S., "A general method for the synthesis of 1,1-difluoroalkylphosphonates." *Tetrahedron Lett.* **1992**, *33*, 1839-42.

130) Martin, S. F.; Wagman, A. S., "A general and efficient route to phosphorodithioate analogs of naturally occurring lipids." *J. Org. Chem.* **1993**, *58*, 5897-9.

131) Aakre, S. E.; Little, C., "Inhibition of Bacillus cereus phospholipase C by univalent anions." *Biochem. J.* **1982**, *203*, 799-801.

132) Amtmann, E., "The antiviral, antitumoural xanthate D609 is a competitive inhibitor of phosphatidylcholine-specific phospholipase C." *Drugs Exp. Clin. Res.* **1996**, *22*, 287-294.

133) Mullerdecker, K., "Interruption of Tpa-Induced Signals by an Antiviral and Antitumoral Xanthate Compound - Inhibition of a Phospholipase C-Type Reaction." *Biochem. Biophys. Res. Commun.* **1989**, *162*, 198-205.

134) Martin, S. F.; Follows, B. C.; Hergenrother, P. J.; Franklin, C. L., "A novel class of zinc-binding inhibitors for the phosphatidylcholine-preferring phospholipase C from Bacillus cereus." *J. Org. Chem.* **2000**, *65*, 4509-4514.

135) Gonzalez-Roura, A.; Navarro, I.; Delgado, A.; Llebaria, A.; Casas, J., "Disclosing new inhibitors by finding similarities in three-dimensional active-site architectures of polynuclear zinc phospholipases and aminopeptidases." *Angew. Chem., Int. Ed. Engl.* **2004**, *43*, 862-865.

136) Piettre, S. R.; Ganzhorn, A.; Hoflack, J.; Islam, K.; Hornsperger, J. M., "alpha-hydroxytropolones: A new class of potent inhibitors of inositol monophosphatase and other bimetallic enzymes." *J. Am. Chem. Soc.* **1997**, *119*, 3201-3204.

137) Beau, J. M.; Gallagher, T., "Nucleophilic C-glycosyl donors for C-glycoside synthesis." In *Glycoscience Synthesis of Substrate Analogs and Mimetics*, 1997; 'Vol.' 187, 1-54.

138) Nicotra, F., "Synthesis of C-glycosides of biological interest." In *Glycoscience Synthesis of Substrate Analogs and Mimetics*, ed.; 1997; 'Vol.' 187, 55-83.

139) Du, Y. G.; Linhardt, R. J.; Vlahov, I. R., "Recent advances in stereoselective C-glycoside synthesis." *Tetrahedron* **1998**, *54*, 9913-9959.

140) Postema, M. H. D., "Recent developments in the synthesis of C-glycosides." *Tetrahedron* **1992**, *48*, 8545-99.

141) Armstrong, R. W.; Beau, J. M.; Cheon, S. H.; Christ, W. J.; Fujioka, H.; Ham, W. H.; Hawkins, L. D.; Jin, H.; Kang, S. H.; et al., "Total synthesis of a fully protected palytoxin carboxylic acid." *J. Am. Chem. Soc.* **1989**, *111*, 7525-30.

142) Kishi, Y., "Natural products synthesis: palytoxin." *Pure Appl. Chem.* **1989**, *61*, 313-24.

143) Armstrong, R. W.; Beau, J. M.; Cheon, S. H.; Christ, W. J.; Fujioka, H.; Ham, W. H.; Hawkins, L. D.; Jin, H.; Kang, S. H.; et al., "Total synthesis of palytoxin carboxylic acid and palytoxin amide." *J. Am. Chem. Soc.* **1989**, *111*, 7530-3.

144) Aicher, T. D.; Buszek, K. R.; Fang, F. G.; Forsyth, C. J.; Jung, S. H.; Kishi, Y.; Matelich, M. C.; Scola, P. M.; Spero, D. M.; Yoon, S. K., "Total synthesis of halichondrin B and norhalichondrin B." *J. Am. Chem. Soc.* **1992**, *114*, 3162-4.

145) Matsumoto, T.; Hosoya, T.; Suzuki, K., "Total synthesis and absolute stereochemical assignment of gilvocarcin M." *J. Am. Chem. Soc.* **1992**, *114*, 3568-70.

146) Hosoya, T.; Takashiro, E.; Matsumoto, T.; Suzuki, K., "Total Synthesis of the Gilvocarcins." *J. Am. Chem. Soc.* **1994**, *116*, 1004-15.

147) Hacksell, U.; Daves, G. D., Jr., "The chemistry and biochemistry of C-nucleosides and C-arylglycosides." *Prog. Med. Chem.* **1985**, *22*, 1-65.

148) Elespuru, R. K.; Gonda, S. K., "Activation of antitumor agent gilvocarcins by visible light." *Science* **1984**, *223*, 69-71.

149) Martin, S. F., "Unified strategy for the synthesis of C-aryl glycosides." *Pure Appl. Chem.* **2003**, *75*, 63-70.

150) Apsel, B.; Bender, J. A.; Escobar, M.; Kaelin, D. E.; Lopez, O. D.; Martin, S. F., "General entries to C-aryl glycosides. Formal synthesis of galtamycinone." *Tetrahedron Lett.* **2003**, *44*, 1075-1077.

151) Matsumoto, T.; Yamaguchi, H.; Suzuki, K., "Total synthesis of galtamycinone, the common aglycon of the C-glycosyl naphthacenequinone antibiotics." *Synlett* **1996**, 433-434.

152) Kanda, N.; Kono, M.; Asano, K., "A new antitumor antibiotic, kidamycin. II. Experimental treatment of cancer with kidamycin." *J. Antibiot.* **1972**, *25*, 553-6.

153) Bennett, G. N., "Formation of alkali labile linkages in DNA by hedamycin and use of hedamycin as a probe of protein-DNA complexes." *Nucleic Acids Res.* **1982**, *10*, 4581-94.

154) Parker, K. A., "Novel methods for the synthesis of C-aryl glycoside natural products." *Pure Appl. Chem.* **1994**, *66*, 2135-8.

155) Postema, M. H. D., "*C-Glycoside Synthesis*."; CRC Press: 1995;.

156) Jaramillo, C.; Knapp, S., "Synthesis of C-aryl glycosides." *Synthesis* **1994**, 1-20.

157) Levy, D. E.; Tang, C., "The Chemistry of *C*-Glycoside." *Tetrahedron Orgainc Chemistry Series* **1995**, *13*.

158) Toshima, K.; Matsuo, G.; Tatsuta, K., "Efficient C-arylglycosylation of 1-O-methyl sugar by novel use of TMSOTf silver perchlorate catalyst system." *Tetrahedron Lett.* **1992**, *33*, 2175-8.

159) Toshima, K.; Matsuo, G.; Ishizuka, T.; Nakata, M.; Kinoshita, M., "C-arylglycosidation of unprotected free sugar." *J. Chem. Soc., Chem. Commun.* **1992**, 1641-2.

160) Toshima, K.; Matsuo, G.; Nakata, M., "An improved practical method for synthesis of aryl C-glycosides from unprotected methyl glycosides and 1-hydroxy sugars." *J. Chem. Soc., Chem. Commun.* **1994**, 997-8.

161) Matsuo, G.; Miki, Y.; Nakata, M.; Matsumura, S.; Toshima, K., "Total synthesis of urdamycinone B via C-glycosidation of an unprotected sugar and Diels-Alder reaction of C-glycosyl juglone." *Chem. Commun.* **1996**, 225-6.

162) Martin, O. R., "The unexpected intramolecular C-arylation of 2-O-benzylated cyclic sugar derivatives: A useful, 1,2-cis-C-glycosylation reaction." *Tetrahedron Lett.* **1985**, *26*, 2055-8.

163) Martin, O. R.; Hendricks, C. A.; Deshpande, P. P.; Cutler, A. B.; Kane, S. A.; Rao, S. P., "Synthesis of C-glycosylarenes by way of internal reactions of benzylated and benzoylated carbohydrate derivatives." *Carbohydr. Res.* **1990**, *196*, 41-58.

164) Martin, O. R.; Rao, S. P.; Hendricks, C. A.; Mahnken, R. E., "Multiple and long-range participation of benzyl groups in intramolecular C-arylation reactions of benzylated glycosides." *Carbohydr. Res.* **1990**, *202*, 49-66.

165) Araki, Y.; Mokubo, E.; Kobayashi, N.; Nagasawa, J.; Ishido, Y., "Structure elucidation and a novel reductive cleavage of the ribofuranosyl ring C(1)-O bond of the intramolecular C-arylation product of tri-O-benzyl-b-D-ribofuranosyl fluoride." *Tetrahedron Lett.* **1989**, *30*, 1115-18.

166) Verlhac, P.; Leteux, C.; Toupet, L.; Veyrieres, A., "Synthesis of 2-(alpha-D-glucopyranosyl)benzoic acid by an intramolecular Friedel-Grafts reaction." *Carbohydr. Res.* **1996**, *291*, 11-20.

167) Matsumoto, T.; Katsuki, M.; Suzuki, K., "New Approach to C-Aryl Glycosides Starting from Phenol and Glycosyl Fluoride - Lewis Acid-Catalyzed Rearrangement of O-Glycoside to C-Glycoside." *Tetrahedron Lett.* **1988**, *29*, 6935-6938.

168) Matsumoto, T.; Hosoya, T.; Suzuki, K., "Improvement in O->C-glycoside rearrangement approach to C-aryl glycosides. Use of 1-O-acetyl sugar as stable but efficient glycosyl donor." *Tetrahedron Lett.* **1990**, *31*, 4629-32.

169) Suzuki, K.; Maeta, H.; Matsumoto, T., "An Improved Procedure for Metallocene-Promoted Glycosidation - Enhanced Reactivity by Employing 1-2-Ratio of Cp2hfcl2-Agclo4." *Tetrahedron Lett.* **1989**, *30*, 4853-4856.

170) Matsumoto, T.; Katsuki, M.; Jona, H.; Suzuki, K., "Synthetic study toward vineomycins. Synthesis of C-aryl glycoside sector via hafnocene dichloride-silver perchlorate-promoted tactics." *Tetrahedron Lett.* **1989**, *30*, 6185-8.

171) Suzuki, K., "Total synthesis of aryl C-glycoside antibiotics." *Pure Appl. Chem.* **1994**, *66*, 2175-8.

172) Matsumoto, T.; Hosoya, T.; Katsuki, M.; Suzuki, K., "New efficient protocol for aryne generation. Selective synthesis of differentially protected 1,4,5-naphthalenetriols." *Tetrahedron Lett.* **1991**, *32*, 6735-6.

173) Futagami, S.; Ohashi, Y.; Imura, K.; Hosoya, T.; Ohmori, K.; Matsumoto, T.; Suzuki, K., "Total synthesis of ravidomycin: revision of absolute and relative stereochemistry." *Tetrahedron Lett.* **2000**, *41*, 1063-1067.

174) Hosoya, T.; Takashiro, E.; Yamamoto, Y.; Matsumoto, T.; Suzuki, K., "Total syntheses of BE-12406 A and its C(8)-vinyl analog." *Heterocycles* **1996**, *42*, 397-414.

175) Matsumoto, T.; Sohma, T.; Yamaguchi, H.; Kurata, S.; Suzuki, K., "Benzyne-furan cycloaddition approach to the angucyclines: first total synthesis of antibiotic C104." *Synlett* **1995**, 263-6.

176) Hosoya, T.; Takashiro, E.; Matsumoto, T.; Suzuki, K., "First total synthesis of BE-12406 A." *Tetrahedron Lett.* **1994**, *35*, 4591-4.

177) Suzuki, K.; Matsumoto, T.; Hosoya, T., "Total synthesis of gilvocarcins as hybrid-type antitumor compounds." *Kagaku to Kogyo* **1993**, *46*, 1103-5.

178) Matsumoto, T.; Yamaguchi, H.; Suzuki, K., "C-Glycosyl juglone in angucycline synthesis: total synthesis of galtamycinone, common aglycon of C-glycosyl naphthacenequinone-type angucyclines." *Tetrahedron* **1997**, *53*, 16533-16544.

179) Matsumoto, T.; Sohma, T.; Yamaguchi, H.; Kurata, S.; Suzuki, K., "Total Synthesis of Antibiotic C104 - Benzyne-Furan Cycloaddition Approach to the Angucyclines." *Tetrahedron* **1995**, *51*, 7347-7360.

180) Suzuki, K., "Synthetic study of ravidomycin, a hybrid natural product." *Pure Appl. Chem.* **2000**, *72*, 1783-1786.

181) Matsumoto, T.; Hosoya, T.; Suzuki, K., "O -> C-Glycoside rearrangement of resorcinol derivatives. Versatile intermediates in the synthesis of aryl C-glycosides." *Synlett* **1991**, 709-11.

182) Boyd, V. A.; Drake, B. E.; Sulikowski, G. A., "Preparation of 2-Deoxy-Beta-C-Arylglycosides and C-Arylglycals from Carbohydrate Lactones." *J. Org. Chem.* **1993**, *58*, 3191-3193.

183) Czernecki, S.; Ville, G., "C-Glycosides. 7. Stereospecific C-glycosylation of aromatic and heterocyclic rings." *J. Org. Chem.* **1989**, *54*, 610-12.

184) Dondoni, A.; Marra, A.; Schermann, M. C., "Furan-based synthesis of C-glycosyl carboxylates." *Tetrahedron Lett.* **1993**, *34*, 7323-6.

185) Ellsworth, B. A.; Doyle, A. G.; Patel, M.; Caceres-Cortes, J.; Meng, W.; Deshpande, P. P.; Pullockaran, A.; Washburn, W. N., "C-Arylglucoside synthesis: triisopropylsilane as a selective reagent for the reduction of an anomeric C-phenyl ketal." *Tetrahedron-Asymmetry* **2003**, *14*, 3243-3247.

186) Boyd, V. A.; Sulikowski, G. A., "Total Synthesis of the Angucycline Antibiotics Urdamycinone-B and 104-2 Via a Common Synthetic Intermediate." *J. Am. Chem. Soc.* **1995**, *117*, 8472-8473.

187) Marzabadi, C. H.; Spilling, C. D., "Stereoselective glucal epoxide formation." *J. Org. Chem.* **1993**, *58*, 3761-6.

188) Halcomb, R. L.; Danishefsky, S. J., "On the Direct Epoxidation of Glycals - Application of a Reiterative Strategy for the Synthesis of Beta-Linked Oligosaccharides." *J. Am. Chem. Soc.* **1989**, *111*, 6661-6666.

189) Klein, L. L.; McWhorter, W. W.; Ko, S. S.; Pfaff, K. P.; Kishi, Y.; Uemura, D.; Hirata, Y., "Stereochemistry of Palytoxin .1. C85-C115 Segment." *J. Am. Chem. Soc.* **1982**, *104*, 7362-7364.

190) Xue, S.; Han, K.-Z.; He, L.; Guo, Q.-X., "Zinc-mediated synthesis of a-C-glycosides from 1,2-anhydro-glycosides." *Synlett* **2003**, 870-872.

191) Allwein, S. P.; Cox, J. M.; Howard, B. E.; Johnson, H. W. B.; Rainier, J. D., "C-Glycosides to fused polycyclic ethers." *Tetrahedron* **2002**, *58*, 1997-2009.

192) Bellosta, V.; Czernecki, S., "Stereocontrolled synthesis of C-glycosides by reaction of organocuprates with protected 1,2-anhydro sugars, and their transformation into 2-deoxy-C-glycosides." *J. Chem. Soc., Chem. Commun.* **1989**, 199-200.

193) Bellosta, V.; Czernecki, S., "C-glycosyl compounds. Part X. Reaction of organocuprate reagents with protected 1,2-anhydro sugars. Stereocontrolled synthesis of 2-deoxy-C-glycosyl compounds." *Carbohydr. Res.* **1993**, *244*, 275-84.

194) Evans, D. A.; Trotter, B. W.; Cote, B., "Addition of allylstannanes to glycal epoxides. a diastereoselective approach to b-C-glycosidation." *Tetrahedron Lett.* **1998**, *39*, 1709-1712.

195) Rainier, J. D.; Cox, J. M., "Aluminum- and Boron-Mediated C-Glycoside Synthesis from 1,2-Anhydroglycosides." *Org. Lett.* **2000**, *2*, 2707-2709.

196) Ferrier, R. J., "Substitution-with-allylic-rearrangement reactions of glycal derivatives." In *Glyoscience: Epimerisation, Isomerisation and Rearrangement Reactions of Carbohydrates*, ed.; 2001; 'Vol.' 215, 153-175.

197) Ferrier, R. J.; Zubkov, O. A., "Transformation of Glycals into 2.3-Unsaturated Glycosyl Derivatives." *Organic Reactions* **2003**, *62*, 569-736.

198) Casiraghi, G.; Cornia, M.; Rassu, G.; Zetta, L.; Fava, G. G.; Belicchi, M. F., "Stereoselective Arylation of Pyranoid Glycals, Using Bromomagnesium Phenolates - an Entry to 2,3-Unsaturated C-Alpha-Glycopyranosylarenes." *Carbohydr. Res.* **1989**, *191*, 243-251.

199) Casiraghi, G.; Cornia, M.; Rassu, G.; Zetta, L.; Fava, G. G.; Belicchi, M. F., "A Simple Diastereoselective Synthesis of 2',3'-Unsaturated Aryl C-Glucopyranosides." *Tetrahedron Lett.* **1988**, *29*, 3323-3326.

200) Steinhuebel, D. P.; Fleming, J. J.; Du Bois, J., "Stereoselective organozinc addition reactions to 1,2-dihydropyrans for the assembly of complex pyran structures." *Org. Lett.* **2002**, *4*, 293-295.

201) Price, S.; Edwards, S.; Wu, T.; Minehan, T., "Synthesis of C-aryl-Delta(2,3)-glycopyranosides via uncatalyzed addition of triarylindium reagents to glycals." *Tetrahedron Lett.* **2004**, *45*, 5197-5201.

202) Di Bussolo, V.; Caselli, M.; Pineschi, M.; Crotti, P., "New stereoselective beta-C-glycosidation by uncatalyzed 1,4-addition of organolithium reagents to a glycal-derived vinyl oxirane." *Org. Lett.* **2003**, *5*, 2173-2176.

203) Di Bussolo, V.; Caselli, M.; Romano, M. R.; Pineschi, M.; Crotti, P., "Stereospecific Uncatalyzed a-O-Glycosylation and a-C-Glycosidation by Means of a New D-Gulal-Derived a Vinyl Oxirane." *J. Org. Chem.* **2004**, *69*, 7383-7386.

204) Bellosta, V.; Czernecki, S., "C-Glycosyl Compounds .4. Synthesis of (2-Deoxy-Alpha-D-Glyc-2-Enopyranosyl)Arenes by Stereospecific Conjugate-Addition of Organocopper Reagents to Peracetylated Hex-1-Enopyran-3-Uloses." *Carbohydr. Res.* **1987**, *171*, 279-288.

205) Benhaddou, R.; Czernecki, S.; Ville, G., "Palladium-Mediated Arylation of Acetylated Enones Derived from Glycals .4. Synthesis of Aryl 2-Deoxy-Beta-D-C-Glycopyranosides." *J. Org. Chem.* **1992**, *57*, 4612-4616.

206) Ramnauth, J.; Poulin, O.; Bratovanov, S. S.; Rakhit, S.; Maddaford, S. P., "Stereoselective C-glycoside formation by a rhodium(I)-catalyzed 1,4-addition of arylboronic acids to acetylated enones derived from glycals." *Org. Lett.* **2001**, *3*, 2571-2573.

207) Cossy, J.; Rakotoarisoa, H., "Organocopper reagents (RCu, R2CuLi) for the nucleophilic C-glycosylation of a C(2)- formyl glycal." *Synlett* **2000**, 734-736.

208) Lesimple, P.; Beau, J. M.; Sinay, P., "Stereocontrolled preparation of 2-deoxy-C-a- and b-D-glucopyranosyl compounds from tributyl(2-deoxy-a- and -b-D-glucopyranosyl)stannanes." *Carbohydr. Res.* **1987**, *171*, 289-300.

209) Cohen, T.; Lin, M. T., "Two-flask preparation of a-lithio cyclic ethers from g- and d-lactones. Reductive lithiation as a route, via radical intermediates, to axial 2-lithiotetrahydropyrans and their equilibration to the equatorial isomers." *J. Am. Chem. Soc.* **1984**, *106*, 1130-1.

210) Lesimple, P.; Beau, J. M.; Sinay, P., "Stereospecific generation of a- and b-glycosyllithium reagents from glycosylstannanes: a stereocontrolled route to a- and b-C-glycosides." *J. Chem. Soc., Chem. Commun.* **1985**, 894-5.

211) Burkhart, F.; Hoffmann, M.; Kessler, H., "C-glycosides: A stereoselective synthesis of alpha- and beta-C-galactosides with glycosyl dianions." *Tetrahedron Lett.* **1998**, *39*, 7699-7702.

212) Wittman, V.; Kessler, H., "Stereoselective Synthesis of C-Glycosides with a Glycosyl Dianion." *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1091-1093.

213) Parker, K. A.; Koh, Y.-h., "Methodology for the Regiospecific Synthesis of Bis C-Aryl Glycosides. Models for Kidamycins." *J. Am. Chem. Soc.* **1994**, *116*, 11149-50.

214) Parker, K. A.; Coburn, C. A., "Reductive aromatization of quinol ketals: a new synthesis of C-aryl glycosides." *J. Am. Chem. Soc.* **1991**, *113*, 8516-18.

215) Parker, K. A.; Coburn, C. A.; Koh, Y.-h., "Reductive and Nonreductive Aromatization of Quinol Ketal Glycals. Models for the Preparation of C-Aryl Glycoside Natural Products." *J. Org. Chem.* **1995**, *60*, 2938-41.

216) Parker, K. A.; Coburn, C. A.; Johnson, P. D.; Aristoff, P., "Reductive aromatization of quinols. New convenient methods for the regiospecific synthesis of p-hydroxy C-aryl glycals." *J. Org. Chem.* **1992**, *57*, 5547-50.

217) Dubois, E.; Beau, J. M., "Synthesis of C-glycopyranosyl compounds by a palladium-catalyzed coupling reaction of 1-tributylstannyl-D-glucals with organic halides." *Carbohydr. Res.* **1992**, *228*, 103-20.

218) Lee, T. D.; Daves, G. D., Jr., "Palladium-mediated reaction of enol ethers with organomercuric acetates." *J. Org. Chem.* **1983**, *48*, 399-402.

219) Outten, R. A.; Daves, G. D., Jr., "Benzo[d]naphtho[1,2-b]pyran-6-one C-glycosides. Aryltri-n-butylstannanes in palladium-mediated coupling with 2,3-dihydropyran and furanoid glycals." *J. Org. Chem.* **1989**, *54*, 29-35.

220) Outten, R. A.; Daves, G. D., Jr., "Synthetic 1-methoxybenzo[d]naphtho[1,2-b]pyran-6-one C-glycosides." *J. Org. Chem.* **1987**, *52*, 5064-6.

221) Farr, R. N.; Outten, R. A.; Cheng, J. C. Y.; Daves, G. D., "C-Glycoside Synthesis by Palladium-Catalyzed Iodoaglycon Glycal Coupling." *Organometallics* **1990**, *9*, 3151-3156.

222) Ramnauth, J.; Poulin, O.; Rakhit, S.; Maddaford, S. P., "Palladium(II) Acetate Catalyzed Stereoselective C-Glycosidation of Peracetylated Glycals with Arylboronic Acids." *Org. Lett.* **2001**, *3*, 2013-2015.

223) Schmidt, B., "Heck arylation of cyclic enol ethers with aryldiazonium salts: regio- and stereoselective synthesis of arylated oxacycles." *Chem. Commun.* **2003**, 1656-1657.

224) Friesen, R. W.; Sturino, C. F., "The Preparation of C-Arylglycals - the Palladium-Catalyzed Coupling of 3,4,6-Tri-O-(Tert-Butyldimethylsilyl)-1-(Tributylstannyl)-D-Glucal and Aryl Bromides." *J. Org. Chem.* **1990**, *55*, 2572-2574.

225) Dubois, E.; Beau, J. M., "Formation of C-Glycosides by a Palladium-Catalyzed Coupling Reaction of Tributylstannyl Glycals with Organic Halides." *J. Chem. Soc., Chem. Commun.* **1990**, 1191-1192.

226) Tius, M. A.; Gu, X. Q.; Gomezgaleno, J., "Convergent Synthesis of Vineomycinone B2 Methyl-Ester." *J. Am. Chem. Soc.* **1990**, *112*, 8188-8189.

227) Tius, M. A.; Gomezgaleno, J.; Gu, X. Q.; Zaidi, J. H., "C-Glycosylanthraquinone Synthesis - Total Synthesis of Vineomycinone-B2 Methyl-Ester." *J. Am. Chem. Soc.* **1991**, *113*, 5775-5783.

228) Friesen, R. W.; Daljeet, A. K., "Hydroboration of C-Arylglucals - Synthesis of the Beta-C-Arylglucoside Nucleus of Chaetiacandin." *Tetrahedron Lett.* **1990**, *31*, 6133-6136.

229) Dubois, E.; Beau, J. M., "Arylation of 1-Tributylstannyl Glycals Catalyzed by Palladium - a Synthetic Route to the Basic Skeleton of the Papulacandins and Chaetiacandin." *Tetrahedron Lett.* **1990**, *31*, 5165-5168.

230) Friesen, R. W.; Loo, R. W., "Preparation of C-Aryl Glucals Via the Palladium-Catalyzed Coupling of Metalated Aromatics with 1-Iodo-3,4,6-Tri-O-(Triisopropylsilyl)-D-Glucal." *J. Org. Chem.* **1991**, *56*, 4821-4823.

231) Dubbaka, S. R.; Steunenberg, P.; Vogel, P., "Aryl and arylmethyl C-glycosides through desulfitative stille and carbonylative stille cross-coupling of tinglycals and sulfonyl chlorides." *Synlett* **2004**, 1235-1238.

232) Lehmann, U.; Awasthi, S.; Minehan, T., "Palladium-Catalyzed Cross-Coupling Reactions between Dihydropyranylindium Reagents and Aryl Halides. Synthesis of C-Aryl Glycals." *Org. Lett.* **2003**, *5*, 2405-2408.

233) Dunkerton, L. V.; Euske, J. M.; Serino, A. J., "Palladium-Assisted Reactions .3. Palladium(0)-Assisted Synthesis of C-Glycopyranosyl Compounds." *Carbohydr. Res.* **1987**, *171*, 89-107.

234) Moineau, C.; Bolitt, V.; Sinou, D., "Stereospecific Synthesis of Alpha-C-Aryl-Delta(2)-Glycopyranosides and Beta-C-Aryl-Delta(2)-Glycopyranosides from P-Tert-Butylphenyl Alpha-O-Delta(2)-Glycopyranoside Via Grignard-Reagents." *J. Chem. Soc., Chem. Commun.* **1995**, 1103-1104.

235) Moineau, C.; Bolitt, V.; Sinou, D., "Synthesis of alpha- and beta-C-aryl Delta(2)-glycopyranosides from p-tert-butylphenyl Delta(2)-glycopyranosides via Grignard reagents." *J. Org. Chem.* **1998**, *63*, 582-591.

236) Moineau, C.; Bolitt, V.; Sinou, D., "Stereochemistry in palladium- and nickel-catalyzed addition of phenylmagnesium bromide to unsaturated carbohydrates." *J. Organomet. Chem.* **1998**, *567*, 157-162.

237) Danishefsky, S.; Phillips, G.; Ciufolini, M., "A Fully Synthetic Route to the Papulacandins - Stereospecific Spiroacetalization of a C-1-Arylated Methyl Glycoside." *Carbohydr. Res.* **1987**, *171*, 317-327.

238) Schmidt, R. R.; Frick, W.; Haagzeino, B.; Apparao, S., "Denovo-Synthesis of Carbohydrates and Related Natural-Products .28. C-Aryl-Glycosides and 3-Deoxy-2-Glyculosonates Via Inverse Type Hetero-Diels-Alder Reaction." *Tetrahedron Lett.* **1987**, *28*, 4045-4048.

239) Maruoka, K.; Itoh, T.; Shirasaka, T.; Yamamoto, H., "Asymmetric Hetero-Diels-Alder Reaction Catalyzed by Chiral Organo-Aluminum Reagent." *J. Am. Chem. Soc.* **1988**, *110*, 310-312.

240) Gao, Q. Z.; Ishihara, K.; Maruyama, T.; Mouri, M.; Yamamoto, H., "Asymmetric Hetero-Diels-Alder Reaction Catalyzed by Stable and Easily Prepared Cab Catalysts." *Tetrahedron* **1994**, *50*, 979-988.

241) Gao, Q. Z.; Maruyama, T.; Mouri, M.; Yamamoto, H., "Asymmetric Hetero-Diels-Alder Reaction Catalyzed by Stable and Easily Prepared Cab Catalysts." *J. Org. Chem.* **1992**, *57*, 1951-1952.

242) Dossetter, A. G.; Jamison, T. F.; Jacobsen, E. N., "Highly enantio- and diastereoselective hetero-Diels-Alder reactions catalyzed by new chiral tridentate chromium(III) catalysts." *Angew. Chem., Int. Ed.* **1999**, *38*, 2398-2400.

243) Anada, M.; Washio, T.; Shimada, N.; Kitagaki, S.; Nakajima, M.; Shiro, M.; Hashimoto, S., "A new dirhodium(II) carboxamidate complex as a chiral lewis acid catalyst for enantioselective hetero-Diels-Alder reactions." *Angew. Chem., Int. Ed.* **2004**, *43*, 2665-2668.

244) Du, H. F.; Long, J.; Hu, J. Y.; Li, X.; Ding, K. L., "3,3 '-Br-2-BINOL-Zn complex: A highly efficient catalyst for the enantioselective hetero-Diels-Alder reaction." *Org. Lett.* **2002**, *4*, 4349-4352.

245) Johannsen, M.; Jorgensen, K. A., "Asymmetric Hetero-Diels-Alder Reactions and Ene Reactions Catalyzed by Chiral Copper(II) Complexes." *J. Org. Chem.* **1995**, *60*, 5757-5762.

246) Kozikowski, A. P.; Cheng, X. M., "An Efficacious Synthesis of Aryl and Heteroaryl C-Glycosides." *J. Chem. Soc., Chem. Commun.* **1987**, 680-683.

247) Hauser, F. M.; Hu, X. D., "A new route to C-aryl glycosides." *Org. Lett.* **2002**, *4*, 977-978.

248) Khan, A. T.; Ahmed, W.; Schmidt, R. R., "A method for the synthesis of C-(2-deoxy-beta-glycosyl)arenes." *Carbohydr. Res.* **1996**, *280*, 277-286.

249) Matsuda, F.; Kawasaki, M.; Terashima, S., "Synthetic Studies on Nogalamycin Congeners - Total Syntheses of (+)-Nogarene, (+)-7-Con-O-Methylnogarol, and Their Related-Compounds." *Pure Appl. Chem.* **1989**, *61*, 385-388.

250) Kawasaki, M.; Matsuda, F.; Terashima, S., "Synthetic Studies on Nogalamycin Congeners 1 Chiral Synthesis of the Def-Ring System of Nogalamycin." *Tetrahedron* **1988**, *44*, 5695-5711.

251) Kawasaki, M.; Matsuda, F.; Terashima, S., "Chiral Synthesis of the Def-Ring System of Nogalamycin." *Tetrahedron Lett.* **1985**, *26*, 2693-2696.

252) Sharma, G. V. M.; Kumar, K. R.; Sreenivas, P.; Krishna, P. R.; Chorghade, M. S., "Catalytic FeCl3- or Yb(OTf)(3)-mediated synthesis of substituted tetrahydrofurans and C-aryl glycosides from 1,4-diols." *Tetrahedron-Asymmetry* **2002**, *13*, 687-690.

253) Krishna, P. R.; Lavanya, B.; Sharma, G. V. M., "Stereoselective synthesis of C-phenyl D- and L-glycero heptopyranosides." *Tetrahedron-Asymmetry* **2003**, *14*, 419-427.

254) Krishna, P. R.; Lavanya, B.; Ilangovan, A.; Sharma, G. V. M., "Stereoselective synthesis of C-alkyl and functionalised C-alkyl glycosides using 'thiophene' as a masked C-4 synthon." *Tetrahedron-Asymmetry* **2000**, *11*, 4463-4472.

255) Schmidt, B.; Sattelkau, T., "Ring closing metathesis as the key step in the synthesis of furan-substituted c-aryl glycosides." *Tetrahedron* **1997**, *53*, 12991-13000.

256) Schmidt, B., "Epoxide opening reactions of aryl substituted dihydropyran oxides: regio- and stereochemical studies directed towards deoxy-aryl-C-glycosides." *J. Chem. Soc., Perkin Trans. 1* **1999**, 2627-2637.

257) Schmidt, B., "Base-induced rearrangement of dihydropyran oxides: A novel synthesis of cyclic enol ethers with a hydroxy-function in the allylic position." *Tetrahedron Lett.* **1999**, *40*, 4319-4320.

258) Schmidt, B., "A de novo synthesis of 2,6-dideoxy-C-aryl glycosides based on ring closing metathesis and diastereoselective epoxide cleavage/anomerization reactions." *Org. Lett.* **2000**, *2*, 791-794.

259) Schmidt, B.; Wildemann, H., "A synthesis of densely functionalized 2,3-dihydropyrans using ring-closing metathesis and base-induced rearrangements of dihydropyran oxides." *Eur. J. Org. Chem.* **2000**, 3145-3163.

260) Schmidt, B.; Wildemann, H., "Diastereoselective ring-closing metathesis in the synthesis of dihydropyrans." *J. Org. Chem.* **2000**, *65*, 5817-5822.

261) Schmidt, B.; Wildemann, H., "Single and double ring closing metathesis in the formation of dihydropyrans and bisoxacyclic systems with a quaternary centre." *J. Chem. Soc., Perkin Trans. 1* **2000**, 2916-2925.

262) Schmidt, B.; Westhus, M., "Diastereoselectivity in a ring closing metathesis reaction with a remote stereogenic centre leading to quaternary dihydropyrans." *Tetrahedron* **2000**, *56*, 2421-2426.

263) Wildemann, H.; Dunkelmann, P.; Muller, M.; Schmidt, B., "A short olefin metathesis-based route to enantiomerically pure arylated dihydropyrans and alpha,beta-unsaturated delta-valero lactones." *J. Org. Chem.* **2003**, *68*, 799-804.

264) Calimente, D.; Postema, M. H. D., "Preparation of C-1 Glycals via Olefin Metathesis. A Convergent and Flexible Approach to C-Glycoside Synthesis." *J. Org. Chem.* **1999**, *64*, 1770-1771.

265) McDonald, F. E.; Zhu, H. Y. H.; Holmquist, C. R., "Rhodium-Catalyzed Alkyne Cyclotrimerization Strategies for C-Arylglycoside Synthesis." *J. Am. Chem. Soc.* **1995**, *117*, 6605-6606.

266) Yamaguchi, M.; Okuma, T.; Horiguchi, A.; Ikeura, C.; Minami, T., "Total Synthesis of (-)-Urdamycinone-B through Polyketide Condensation." *J. Org. Chem.* **1992**, *57*, 1647-1649.

267) Yamaguchi, M.; Horiguchi, A.; Ikeura, C.; Minami, T., "A Synthesis of Aryl C-Glycosides Via Polyketides." *J. Chem. Soc., Chem. Commun.* **1992**, 434-436.

268) Kaelin, D. E.; Sparks, S. M.; Plake, H. R.; Martin, S. F., "Regioselective synthesis of unsymmetrical C-aryl glycosides using silicon tethers as disposable linkers." *J. Am. Chem. Soc.* **2003**, *125*, 12994-12995.

269) Kaelin, D. E.; Lopez, O. D.; Martin, S. F., "General strategies for the synthesis of the major classes of C-aryl glycosides." *J. Am. Chem. Soc.* **2001**, *123*, 6937-6938.

270) Chen, C. L.; Martin, S. F., "Facile synthesis of 2-substituted 1,2-dihydro-1-naphthols and 2-substituted 1-naphthols." *Org. Lett.* **2004**, *6*, 3581-3584.

271) Anderson, J. E.; Franck, R. W.; Mandella, W. L., "Peri interactions in some 1,8-di-tert-butylnaphthalene compounds. Rotation and flipping of the tert-butyl groups." *J. Am. Chem. Soc.* **1972**, *94*, 4608-14.

272) Kaelin, D. E. "Novel Methodologies for the Synthesis of C-Aryl Glycosides and Progress Toward the Synthesis of the C-Aryl Glycoside Natural Products Galtamycinone and Kidamycin." The University Of Texas at Austin, Austin, 2002.

273) Omura, S.; Tanaka, H.; Oiwa, R.; Awaya, J.; Masuma, R.; Tanaka, K., "New antitumor antibiotics, OS-4742 A1, A2, B1 and B2 produced by a strain of Streptomyces." *J. Antibiot.* **1977**, *30*, 908-16.

274) Imamura, N.; Kakinuma, K.; Ikekawa, N.; Tanaka, H.; Omura, S., "The structure of vineomycin B2." *J. Antibiot.* **1981**, *34*, 1517-8.

275) Bolitt, V.; Mioskowski, C.; Kollah, R. O.; Manna, S.; Rajapaksa, D.; Falck, J. R., "Total synthesis of vineomycinone B2 methyl ester via double Bradsher cyclization." *J. Am. Chem. Soc.* **1991**, *113*, 6320-1.

276) Danishefsky, S.; Uang, B. J.; Quallich, G., "Total synthesis of vineomycinone B2 methyl ester." *J. Am. Chem. Soc.* **1985**, *107*, 1285-93.

277) Matsumoto, T.; Katsuki, M.; Jona, H.; Suzuki, K., "Convergent total synthesis of vineomycinone B2 methyl ester and its C(12)-epimer." *J. Am. Chem. Soc.* **1991**, *113*, 6982-92.

278) Sparks, S. M., "Studies Toward the Total Synthesis of Vineomycinone B2 Methyl Ester." *Final Report* **2003**.

279) Hergenrother, P. J.; Spaller, M. R.; Martin, S. F., "Exploring the catalytic mechanism of phospholipase C." *Abs. Pap. Am. Chem. Soc.* **1997**, *213*, 21-ORGN.

280) Martin, S. F.; Follows, B. C.; Hergenrother, P. J.; Trotter, B. K., "The choline binding site of phospholipase C (Bacillus cereus): Insights into substrate specificity." *Biochemistry* **2000**, *39*, 3410-3415.

281) Antikainen, N. M.; Hergenrother, P. J.; Harris, M. M.; Corbett, W.; Martin, S. F., "Altering substrate specificity of phosphatidylcholine-preferring phospholipase C of Bacillus cereus by random mutagenesis of the headgroup binding site." *Biochemistry* **2003**, *42*, 1603-1610.

282) Martin, S. F.; Spaller, M. R.; Hergenrother, P. J., "Expression and site-directed mutagenesis of the phosphatidylcholine-preferring phospholipase C of Bacillus cereus: Probing the role of the active site Glu146." *Biochemistry* **1996**, *35*, 12970-12977.

283) Wiseman, T.; Williston, S.; Brandts, J. F.; Lin, L. N., "Rapid Measurement of Binding Constants and Heats of Binding Using a New Titration Calorimeter." *Anal. Biochem.* **1989**, *179*, 131-137.

284) Hergenrother, P. J.; Spaller, M. R.; Haas, M. K.; Martin, S. F., "Chromogenic Assay for Phospholipase-C from Bacillus-Cereus." *Anal. Biochem.* **1995**, *229*, 313-316.

285) Hergenrother, P. J.; Haas, M. K.; Martin, S. F., "Chromogenic assay for phospholipase D from Streptomyces chromofuscus: Application to the evaluation of substrate analogs." *Lipids* **1997**, *32*, 783-788.

286) Snyder, W. R., "Bacillus-Cereus Phospholipase-C - Carboxylic-Acid Ester Specificity and Stereoselectivity." *Biochim. Biophys. Acta* **1987**, *920*, 155-160.

287) Franklin, C. L.; Li, H.; Martin, S. F., "Design, Synthesis, and Evaluation of Water-Soluble Phospholipid Analogues as Inhibitors of Phospholipase C from Bacillus cereus." *J. Org. Chem.* **2003**, *68*, 7298-7307.

288) Horita, K.; Yoshioka, T.; Tanaka, T.; Oikawa, Y.; Yonemitsu, O., "On the Selectivity of Deprotection of Benzyl, Mpm (4-Methoxybenzyl) and Dmpm (3,4-Dimethoxybenzyl) Protecting Groups for Hydroxy Functions." *Tetrahedron* **1986**, *42*, 3021-3028.

289) Veysoglu, T.; Mitscher, L. A.; Swayze, J. K., "A Convenient Method for the Control of Selective Ozonizations of Olefins." *Synthesis* **1980**, 807-810.

290) Rastetter, W. H.; Phillion, D. P., "Template-Driven Macrolide Closures." *J. Org. Chem.* **1981**, *46*, 3209-3214.

291) Schreiber, S. L.; Claus, R. E.; Reagan, J., "Ozonolytic Cleavage of Cycloalkenes to Terminally Differentiated Products." *Tetrahedron Lett.* **1982**, *23*, 3867-3870.

292) Frank, R.; Schutkowski, M., "Extremely mild reagent for Boc deprotection applicable to the synthesis of peptides with thioamide linkages." *Chem. Commun.* **1996**, 2509-2510.

293) Valencic, M.; van der Does, T.; de Vroom, E., "Titanium tetrachloride promoted hydrolysis of cephalosporin tert-butyl esters." *Tetrahedron Lett.* **1998**, *39*, 1625-1628.

294) Mehta, A.; Jaouhari, R.; Benson, T. J.; Douglas, K. T., "Improved Efficiency and Selectivity in Peptide-Synthesis - Use of Triethylsilane as a Carbocation Scavenger in Deprotection of Tert-Butyl Esters and Tert-Butoxycarbonyl-Protected Sites." *Tetrahedron Lett.* **1992**, *33*, 5441-5444.

295) Sher, P. M.; Stein, P. D.; Floyd, D.; Hall, S. E. "Preparation of 7-oxabicycloheptyl-substituted azolecarboxamide prostaglandin analogs useful in the treatment of thrombotic and vasospastic disease." 89-123799
374952, 19891222., 1990.

296) Briggs, G. E.; Haldane, J. B. S., "Note on the kinetics of enzyme action." *Biochem. J.* **1925**, *19*, 338-9.

297) Michaelis, L.; Menten, M. L., "Kinetics of Invertase Action." *Biochemische Zeitschrift* **1913**, *49*, 333-69.

298) Lineweaver, H.; Burk, D., "Determination of enzyme dissociation constants." *J. Am. Chem. Soc.* **1934**, *56*, 658-66.

299) Antikainen, N. M. "Altering and Examining the Substrate Specificity of Phospholipase C from Bacillus cereus." The University of Texas at Austin, 2003.

300) Little, C., "Phospholipase C from Bacillus cereus. Action on some artificial lecithins." *Acta Chemica Scandinavica, Series B: Organic Chemistry and Biochemistry* **1977**, *B31*, 267-72.

301) Benfield, A., "unpublished results." **2004**.

302) Eshun, K.; He, Q., "Aloe vera: A valuable ingredient for the food, pharmaceutical and cosmetic industries - A review." *Critical Reviews in Food Science and Nutrition* **2004**, *44*, 91-96.

303) Dagne, E.; Bisrat, D.; Viljoen, A.; Van Wyk, B. E., "Chemistry of Aloe species." *Curr. Org. Chem.* **2000**, *4*, 1055-1078.

304) Reynolds, T.; Dweck, A. C., "Aloe vera leaf gel: a review update." *Journal of Ethnopharmacology* **1999**, *68*, 3-37.

305) Sefkow, M.; Kaatz, H., "Selective protection of either the phenol or the hydroxy group in hydroxyalkyl phenols." *Tetrahedron Lett.* **1999**, *40*, 6561-6562.

306) Overkleeft, H. S.; Vanwiltenburg, J.; Pandit, U. K., "A Facile Transformation of Sugar Lactones to Azasugars." *Tetrahedron* **1994**, *50*, 4215-4224.

307) Gronowitz, S.; Hornfeldt, A. B.; Pettersson, K., "Synthesis of chloro derivatives of five-membered heterocyclics via the lithium derivatives." *Synth. Commun.* **1973**, *3*, 213-18.

308) Politis, J. K.; Nemes, J. C.; Curtis, M. D., "Synthesis and Characterization of Regiorandom and Regioregular Poly(3-octylfuran)." *J. Am. Chem. Soc.* **2001**, *123*, 2537-2547.

309) Allen, J. M.; Aprahamian, S. L.; Sans, E. A.; Shechter, H., "Electronic effects and the stereochemistries in rearrangement-displacement reactions of triaryl(halomethyl)silanes with fluoride and with alkoxide ions." *J. Org. Chem.* **2002**, *67*, 3561-3574.

310) Achmatowicz, B.; Jankowski, P.; Wicha, J.; Zarecki, A., "Migration of aryl groups from silicon to carbon in alpha,beta-epoxysilanes. A new model for hypervalent silicon study." *J. Organomet. Chem.* **1998**, *558*, 227-230.

311) Hudrlik, P. F.; Abdallah, Y. M.; Hudrlik, A. M., "Rearrangements of Alpha-Halosilanes Induced by Intramolecular Nucleophilic-Attack at Silicon." *Tetrahedron Lett.* **1992**, *33*, 6743-6746.

312) Hudrlik, P. F.; Abdallah, Y. M.; Hudrlik, A. M., "Generation of Anionic Intermediates by Intramolecular Nucleophilic-Attack at Silicon." *Tetrahedron Lett.* **1992**, *33*, 6747-6750.

313) Kreeger, R. L.; Menard, P. R.; Sans, E. A.; Shechter, H., "Marked Medium Effects on the Substitution and the Addition-Rearrangement-Ejection Reactions of (Halomethyl)Silanes with Methoxides." *Tetrahedron Lett.* **1985**, *26*, 1115-1118.

314) Sans, E. A.; Shechter, H., "The Behavior of Alkoxides with Allyl(Chloromethyl)Dimethylsilanes and (Chloromethyl)Dimethylvinylsilane - the Abilities of Allyl and Vinyl Groups to Migrate from Pentacoordinate Silicon." *Tetrahedron Lett.* **1985**, *26*, 1119-1122.

315) Tsunoda, T.; Yamamiya, Y.; Ito, S., "1,1'-(Azodicarbonyl)Dipiperidine-Tributylphosphine, a New Reagent System for Mitsunobu Reaction." *Tetrahedron Lett.* **1993**, *34*, 1639-1642.

316) Gassmann, S.; Guintchin, B.; Bienz, S., "New silicon groups as potential chiral auxiliaries. Synthesis and highly selective chiral 1,6-induction in 1,2-additions to acylsilanes." *Organometallics* **2001**, *20*, 1849-1859.

317) Bedford, R. B.; Cazin, C. S. J.; Holder, D., "The development of palladium catalysts for C-C and C-heteroatom bond forming reactions of aryl chloride substrates." *Coord. Chem. Rev.* **2004**, *248*, 2283-2321.

318) Wolter, M.; Nordmann, G.; Job, G. E.; Buchwald, S. L., "Copper-catalyzed coupling of aryl iodides with aliphatic alcohols." *Org. Lett.* **2002**, *4*, 973-976.

319) Torraca, K. E.; Huang, X. H.; Parrish, C. A.; Buchwald, S. L., "An efficient intermolecular palladium-catalyzed synthesis of aryl ethers." *J. Am. Chem. Soc.* **2001**, *123*, 10770-10771.

320) Giles, R. G. F.; Sargent, M. V.; Sianipar, H., "Regioselectivity in the Reactions of Methoxydehydrobenzenes with Furans .1. Reactions of 3-Methoxydehydrobenzene and 3-(Methoxycarbonyl)-Dehydrobenzene with 2-Substituted Furans." *J. Chem. Soc., Perkin Trans. 1* **1991**, 1571-1579.

321) Giles, R. G. F.; Hughes, A. B.; Sargent, M. V., "Regioselectivity in the Reactions of Methoxydehydrobenzenes with Furans .2. 2-Methoxyfuran and Methoxydehydrobenzenes." *J. Chem. Soc., Perkin Trans. 1* **1991**, 1581-1587.

322) Baker, R. W.; Baker, T. M.; Birkbeck, A. A.; Giles, R. G. F.; Sargent, M. V.; Skelton, B. W.; White, A. H., "Regioselectivity in the Reactions of Methoxydehydrobenzenes with Furans .3. 3-Methoxyfuran and Methoxydehydrobenzenes and the Chemistry of Their Adducts." *J. Chem. Soc., Perkin Trans. 1* **1991**, 1589-1600.

323) Gnaim, J. M.; Sheldon, R. A., "Highly Regioselective Ortho-Chlorination of Phenol with Sulfuryl Chloride in the Presence of Amines." *Tetrahedron Lett.* **1995**, *36*, 3893-3896.

324) Lulinski, S.; Serwatowski, J., "Bromine as the ortho-directing group in the aromatic metalation/silylation of substituted bromobenzenes." *J. Org. Chem.* **2003**, *68*, 9384-9388.

325) Iwao, M., "Directed Lithiation of Chlorobenzenes - Regioselectivity and Application to a Short Synthesis of Benzocyclobutenes." *J. Org. Chem.* **1990**, *55*, 3622-3627.

326) Johnson, C. R.; Medich, J. R., "Efficient Preparation of [(Methoxymethoxy)Methyl]Tributylstannane, a Convenient Hydroxymethyl Anion Equivalent." *J. Org. Chem.* **1988**, *53*, 4131-4133.

327) McCann, G. M.; McDonnell, C. M.; Magris, L.; O'Ferrall, R. A. M., "Enol-keto tautomerism of 9-anthrol and hydrolysis of its methyl ether." *Journal of the Chemical Society-Perkin Transactions 2* **2002**, 784-795.

328) Meyers, A. I.; Babiak, K. A.; Campbell, A. L.; Comins, D. L.; Fleming, M. P.; Henning, R.; Heuschmann, M.; Hudspeth, J. P.; Kane, J. M.; et al., "Total synthesis of (-)-maysine." *J. Am. Chem. Soc.* **1983**, *105*, 5015-24.

329) Kane, J. M.; Meyers, A. I., "Progress toward the total synthesis of maytansinoids. A facile route to the aromatic moiety (western zone)." *Tetrahedron Lett.* **1977**, 771-4.

330) Aidhen, I. S.; Ahuja, J. R., "A Novel Synthesis of Benzocyclobutenones." *Tetrahedron Lett.* **1992**, *33*, 5431-5432.

331) Bailey, W. F.; Patricia, J. J.; Nurmi, T. T.; Wang, W., "Metal-Halogen Interchange between Tert-Butyllithium and 1-Iodo-5-Hexenes Provides No Evidence for Single-Electron Transfer." *Tetrahedron Lett.* **1986**, *27*, 1861-1864.

332) Acheson, R. M., "An Introduction to the Chemistry of Heterocyclic Compounds." 1976, 136.

333) Kotsuki, H.; Kondo, A.; Nishizawa, H.; Ochi, M.; Matsuoka, K., "High-Pressure Diels-Alder Reactions of Vinylfurans." *J. Org. Chem.* **1981**, *46*, 5454-5455.

334) Friesen, R. W.; Loo, R. W.; Sturino, C. F., "The Preparation of C-Aryl Glucals Via Palladium-Catalyzed Cross-Coupling Methods." *Canadian Journal of Chemistry-Revue Canadienne De Chimie* **1994**, *72*, 1262-1272.

335) Friesen, R. W.; Sturino, C. F.; Daljeet, A. K.; Kolaczewska, A., "Observation of Alpha-Silyl Carbanions in the Metalation of 3,4,6-Tri-O-(Tert-Butyldimethylsilyl)-D-Glucal." *J. Org. Chem.* **1991**, *56*, 1944-1947.

336) Lesimple, P.; Beau, J. M.; Jaurand, G.; Sinay, P., "Preparation and Use of Lithiated Glycals - Vinylic Deprotonation Versus Tin-Lithium Exchange from 1-Tributylstannyl Glycals." *Tetrahedron Lett.* **1986**, *27*, 6201-6204.

337) Paquette, L. A.; Oplinger, J. A., "Synthesis of a Structurally Modified Glycal - (-)-(2r,4s)-2-Methyl-2-Vinyl-4-(Benzyloxy)-3,4-Dihydro-2h-Pyran." *J. Org. Chem.* **1988**, *53*, 2953-2959.

338) Schmidt, R. R.; Preuss, R.; Betz, R., "Vinyl Carbanions .33. C-1 Lithiation of C-2 Activated Glucals." *Tetrahedron Lett.* **1987**, *28*, 6591-6594.

339) Nicolaou, K. C.; Shi, G. Q.; Gunzner, J. L.; Gartner, P.; Yang, Z., "Palladium-catalyzed functionalization of lactones via their cyclic ketene acetal phosphates. Efficient new synthetic technology for the construction of medium and large cyclic ethers." *J. Am. Chem. Soc.* **1997**, *119*, 5467-5468.

340) Nicolaou, K. C.; Gunzner, J. L.; Shi, G. Q.; Agrios, K. A.; Gartner, P.; Yang, Z., "Total synthesis of brevetoxin A: Part 4: Final stages and completion." *Chemistry-a European Journal* **1999**, *5*, 646-658.

341) Sasaki, M.; Noguchi, K.; Fuwa, H.; Tachibana, K., "Convergent synthesis of an HIJK ring model of ciguatoxin via Suzuki cross-coupling reaction." *Tetrahedron Lett.* **2000**, *41*, 1425-1428.

342) Lepifre, F.; Buon, C.; Rabot, R.; Bouyssou, P.; Coudert, G., "Palladium-catalysed coupling of vinyl phosphates with aryl or heteroaryl boronic acids. Application to the synthesis of substituted nitrogen containing heterocycles." *Tetrahedron Lett.* **1999**, *40*, 6373-6376.

343) Kolakowski, J. G. "Studies Toward the Hydroboration-Oxidation and Hydroboration-Amination of C-Aryl Glycals." The University Of Texas at Austin, Austin, 2003.

344) Dahanukar, V. H.; Rychnovsky, S. D., "General synthesis of alpha-acetoxy ethers from esters by DIBALH reduction and acetylation." *J. Org. Chem.* **1996**, *61*, 8317-8320.

345) Kopecky, D. J.; Rychnovsky, S. D., "Improved procedure for the reductive acetylation of acyclic esters and a new synthesis of ethers." *J. Org. Chem.* **2000**, *65*, 191-198.

346) Hojo, M.; Masuda, R.; Sakaguchi, S.; Takagawa, M., "A Convenient Synthetic Method for Beta-Alkoxyacrylic and Beta-Phenoxyacrylic Acids and 3,4-Dihydro-2h-Pyran-5-Carboxylic and 2,3-Dihydrofuran-4-Carboxylic Acids." *Synthesis* **1986**, 1016-1017.

347) Pocker, Y.; Green, E., "Mechanism of Aminolysis of Delta-Lactones - Kinetic-Behavior of Tri-O-Methyl-2-Deoxyglucono-Delta-Lactone, Solvent Deuterium-Isotope Effects, and Transition-State Characterization." *J. Am. Chem. Soc.* **1976**, *98*, 6197-6202.

348) Yang, W. B.; Chang, C. F.; Wang, S. H.; Teo, C. F.; Lin, C. H., "Expeditious synthesis of C-glycosyl conjugated dienes and aldehydes from sugar lactones." *Tetrahedron Lett.* **2001**, *42*, 4657-4660.

349) Yang, W. B.; Yang, Y. Y.; Gu, Y. F.; Wang, S. H.; Chang, C. C.; Lin, C. H., "Stereochemistry in the synthesis and reaction of exo-glycals." *J. Org. Chem.* **2002**, *67*, 3773-3782.

350) Procko, K. "*C-Aryl Glucal Studies*;" University of Texas a Austin: Austin, 2005.

351) Still, W. C.; Kahn, M.; Mitra, A., "Rapid Chromatographic Technique for Preparative Separations with Moderate Resolution." *J. Org. Chem.* **1978**, *43*, 2923-2925.

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